Relationship between Molecular Weight of T4 Phage-induced Deoxynucleotide Kinase and the Size of Its Messenger Ribonucleic Acid*

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SUMMARY

T4 phage-induced deoxynucleotide kinase was purified to homogeneity as revealed by polyacrylamide gel electrophoresis. The molecular weight of this enzyme was estimated to be about 52,000 by the technique of Sephadex G-200 column chromatography. Enzyme denatured with 2-mercaptoethanol and sodium dodecyl sulfate also showed a single band after electrophoresis in an acrylamide gel containing sodium dodecyl sulfate. In the latter case, the molecular weight was calculated to be approximately 23,000, suggesting that the native enzyme is composed of two identical subunits.

The messenger RNA for deoxynucleotide kinase produced in vivo has been found to be present in two relatively sharp bands that sediment in sucrose density gradients with a coefficient of 15 S and 12 S. This RNA, as well as unfractionated RNA, could direct in vitro the synthesis of deoxynucleotide kinase. The molecular weight of the enzyme synthesized in vitro was determined by gel filtration chromatography and found to be 48,000, approximately the same value as that found for the in vivo enzyme.

The kinase messenger in 12 S RNA is believed to be transcribed from T4 DNA as a monocistronic unit. However, kinase messenger also appears in di- or polycistronic form since 15 S RNA is large enough to code for the synthesis of the kinase and at least one other protein.

Several reports have appeared concerning the size of messenger RNA synthesized in vivo after infection of Escherichia coli with T-even bacteriophage. Adesnik and Levinthal (1) have shown by the use of polyacrylamide gel electrophoresis of pulse-labeled T4 RNA that the molecular weight of T4 RNA is distributed between the range of 2.5 × 10⁴ to 1.5 × 10⁵. However, none of these RNA species was related to specific proteins. Volkin and Riggsby (2) have attempted to correlate the chain lengths of T2 mRNA of unknown function with the size of some T2 phage-induced proteins. They obtained values between 135 and 800 for the nucleotide chain lengths when RNAs were separated by chromatography on a column of methylated albumin coated kieselguhr followed by centrifugation on sucrose density gradients. Their values for the chain lengths of mRNA, however, were too small to correspond to proteins in the molecular weight range of 50,000 unless these proteins were composed of subunits.

Studies have also been carried out attempting to relate the size of individual messenger RNAs formed during T4 infection of E. coli to the molecular weight of their specific protein products. T4-induced lysozyme mRNA has been reported to be 3- to 4-fold larger than that expected from the molecular weight of the lysozyme molecule (3). These findings indicate that the lysozyme messenger RNA is polyribosomatic or is aggregated with other mRNAs or rRNA, or both.

We have previously reported the synthesis of T4 deoxynucleotide kinase (EC 2.7.4.4) in vitro programmed by T4 RNA in the presence of an E. coli cell-free extract (4). Upon centrifugation in a sucrose density gradient, T4 kinase mRNA was separated into two peaks, corresponding to sedimentation coefficients of 15 S and 12 S. We did not know at that time the molecular weight of T4-induced deoxynucleotide kinase. In the present study, we present evidence relating the size of this specific mRNA and the molecular weight of its product, deoxynucleotide kinase.

MATERIALS AND METHODS

Purification of T4-Deoxynucleotide Kinase Synthesized in Vivo—E. coli B cells were grown in 3XD media (5) and infected with T4am82 for 30 min. A total of 100 g wet weight of infected cells thus obtained was used as starting material. The purification of deoxynucleotide kinase was carried out by a modification of the procedure of Duckworth and Beman (6). A unit of enzyme activity is defined as the amount of enzyme which catalyzes the phosphorylation of 1 nmole of substrate per min at 37°. The sample of enzyme, prepared by their procedure up to and including fractionation on a column of hydroxylapatite, was concentrated by filtration through Amicon filters (pm 10) and then applied to a Sephadex G-200 gel column. The specific activity of the fraction obtained by gel filtration was about 95,300 units per mg of protein or about 400-fold higher than that of the original crude extract. The analytical gel electrophoresis revealed that this fraction contained only one protein component (Fig. 1a).
Incubation was for 20 min at 37°C. Thereafter, the protein synthesis mixture was fractionated with ammonium sulfate. The fraction between 33 and 55% saturation with respect to ammonium sulfate contained the enzyme activity. The precipitate was dissolved in 1 ml of 0.05 M potassium phosphate buffer (pH 6.5) containing 0.01 M 2-mercaptoethanol, and was then dialyzed against the same buffer for 4 to 6 hours at 4°C. Almost 100% of the original enzyme activity synthesized in vitro was recovered by this procedure.

**Results**

**Molecular Weight of Deoxynucleotide Kinase Formed In Vivo**

The estimation of the molecular weight of deoxynucleotide kinase was carried out by chromatography on a column of Sephadex G-200. In Fig. 2 is shown a plot of log molecular weight of the proteins examined against their $K_d$ values. The molecular weight of native deoxynucleotide kinase was calculated to be about 52,000.

In order to determine whether or not the native deoxynucleotide kinase molecule is composed of one single polypeptide chain, the enzyme was denatured, reduced with 2-mercaptoethanol in the presence of SDS, and analyzed by SDS-gel electrophoresis. Fig. 1 shows the single band obtained after electrophoresis. This polypeptide has a molecular weight of about 52,000.

**Polyacrylamide Gel Electrophoresis**

The enzyme was analyzed by electrophoresis on gel columns of 7% polyacrylamide (0.6 x 10 cm) according to the procedure described by Moyer and Buchanan (9). For the estimation of molecular weight of the denatured enzyme, the procedure of Weber and Osborn (10) was used. Purified enzyme (100 μg) was denatured in 0.1 M sodium phosphate buffer (pH 7.1), containing 1% SDS and 0.1% 2-mercaptoethanol. Electrophoresis was performed at 10 mA for 4 to 5 hours in 7% polyacrylamide gel containing 0.1 M sodium phosphate buffer (pH 7.1) and 0.1% SDS. The electrophoresis buffer was 0.1 M sodium phosphate buffer (pH 7.1) with 0.1% SDS. The enzyme was stained with Coomassie brilliant blue and excess dye was removed electrophoretically.

The log of the known molecular weight of the standard proteins was plotted against their relative mobilities. The molecular weight of the enzyme could then be estimated from its own relative mobility.

**Molecular Weight of Polydeoxynucleotide Kinase**

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**RESULTS**

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In order to determine whether or not the native deoxynucleotide kinase molecule is composed of one single polypeptide chain, the enzyme was denatured, reduced with 2-mercaptoethanol in the presence of SDS, and analyzed by SDS-gel electrophoresis. Fig. 1 shows the single band obtained after electrophoresis. This polypeptide has a molecular weight of about 23,000 as determined from its mobility relative to those of known marker proteins (Fig. 3). From these results we tentatively suggest

1. The abbreviation used is: SDS, sodium dodecyl sulfate.
2. Dr. W. M. Huang (personal communication) has also identified the protein product of gene 1 and determined that it has a molecular weight of approximately 22,000 in SDS-polyacrylamide gel electrophoresis.

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**Fig. 1.** Polyacrylamide gel electrophoresis of purified T4-deoxynucleotide kinase. Fifteen micrograms of native (a) or denatured (b) deoxynucleotide kinase were applied on a 7% acrylamide gel column. The conditions of electrophoresis were as follows. The buffer for the anode chamber contained 45.5 g of Tris base and 2.5 ml of concentrated HCl per liter at a final pH of 8.9. The buffer for the cathode chamber contained 0.6 g of Tris base and 2.88 g of glycine per liter at pH 8.3. Electrophoresis was carried out with a current of 2 ma per gel until the tracking dye reached the bottom of the gel.

**Fig. 2.** Molecular weight of deoxynucleotide kinase as determined by gel filtration on a column of Sephadex G-200. The proteins examined against their $K_d$ values. The molecular weight of deoxynucleotide kinase is estimated from its mobility relative to those of known marker proteins (Fig. 3). From these results we tentatively suggest

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Molecular weight estimation of deoxynucleotide kinase synthesized in vivo or in vitro.

Gel filtration chromatography was carried out on a column (1 × 100 cm) of Sephadex G-200 equilibrated with 0.05 M potassium phosphate buffer (pH 6.5). Each sample was applied in 1 ml of the same buffer. The amount of protein applied was 50 μg in the case of the purified enzyme and 10 mg in the case of each of the marker proteins. In the latter case, absorbance at 280 nm was determined for each fraction. In all of the experiments the flow rate was about 1 ml per 5 min. The calibration line was made by plotting the log of molecular weight of the marker proteins and their Kd values.

Molecular weight estimation of denatured deoxynucleotide kinase by SDS-gel electrophoresis. Electrophoresis was carried out as described under "Materials and Methods." The log of the molecular weight was plotted against relative mobilities. The relative mobility of each protein was calculated by scanning the stained gels with a Gilford densitometer. The protein markers are human immunoglobulin and its heavy and light chains which are designated as follows: HH, dimer of heavy chains; HL, dimer of heavy and light chains; H, heavy chain; L, light chain.

T4-induced deoxynucleotide kinase is composed of two identical polypeptide chains.

mRNA for Deoxynucleotide Kinase—We have shown previously that mRNA for deoxynucleotide kinase is separated into two components (15 S and 12 S) when mRNA was assayed after centrifugation in sucrose density gradients (4). In order to minimize the aggregation of RNA, samples prepared for analysis by this procedure were heated at 70° for 4 min according to the method of Gesteland and Salser (3). The effect of incubation of kinase messenger RNA at different temperatures on its activity is shown in Fig. 4. T4 RNA showed the maximum kinase messenger activity after incubation at 60° for 4 min in the presence of 10 mM Tris-Cl (pH 7.5) and 1 mM EDTA. The RNA specimens thus obtained were analyzed by centrifugation on a sucrose density gradient (Fig. 5, inset). Pooled fractions corresponding to the arrows were compared for their kinase messenger activity with that of unfractionated RNA. 15 S RNA and 12 S RNA have, respectively, 2 and 3 times higher kinase messenger activity per μg of RNA than does unfractionated T4 8-min RNA (Fig. 5).

Estimation of Molecular Weight of Deoxynucleotide Kinase Synthesized in Vitro—The samples described above were used for the synthesis of deoxynucleotide kinase. The enzyme was then concentrated by precipitation with ammonium sulfate, and the dissolved precipitate was dialyzed. In all cases approximately 100% of the activity synthesized was recovered during the concentration procedure. The molecular weight of the enzyme in each sample was estimated by the same method of Sephadex G-200 column chromatography as described for the in vivo enzyme. As an example, the elution pattern of the deoxynucleotide kinase synthesized by unfractionated T4 8-min RNA is shown in Fig. 6. The profiles of both the absorbance at 280 nm and the activity of enzyme, synthesized with either 15 S RNA or 12 S RNA, were also about the same as that shown in Fig. 6. By means of the same standard curve as shown for the in vitro enzyme, we have estimated that the molecular weights of deoxynucleotide kinase synthesized with unfractionated T4 8-min RNA, 15 S RNA, and 12 S RNA are 48,500, 46,500, and 49,500, respectively (Fig. 2).

DISCUSSION

We have shown by sucrose density gradient centrifugation that T4 deoxynucleotide kinase mRNA is separated into two species of different sizes corresponding to sedimentation coefficients of 15 S and 12 S. On the basis of a formula \( M = 1500 S^2 \) proposed by Spirin (11) for the calculation of the molecular weights of RNA from their sedimentation coefficients on sucrose density gradients, we estimate that the molecular weights of these messengers are \( 4.6 \times 10^6 \) and \( 2.9 \times 10^6 \), respectively.
The molecular weight of deoxynucleotide kinase mRNA after centrifugation in a sucrose density gradient is shown in the inset. RNA was incubated at 60° for 4 min under the conditions described in the legend of Fig. 4 at a final concentration of 3 mg per ml. The samples were rapidly cooled. One milliliter of this RNA was layered onto 36 ml of a 5 to 20% linear sucrose gradient in 0.01 M sodium acetate (pH 5.1) and 0.1 M NaCl. Centrifugation was carried out in a SW 27 rotor for 18 hours at 27,000 rpm and 4°. After fractionation, a portion of each fraction (50 μl) was assayed for kinase messenger activity as described in a previous communication (4). Fractions corresponding to numbers 17 through 21 and 22 through 25 were pooled separately and made 0.1 M with respect to sodium acetate buffer (pH 5.1). To each volume of this solution were added 2 volumes of cold ethanol. The precipitate thus obtained was dissolved in distilled water and stored at -20° until used.

Furthermore, if the entire length of the nucleotide chain is used for coding of proteins, one can predict that they would direct the synthesis of proteins with molecular weights of 43,000 and 27,000, respectively. In this calculation the average molecular weights of a nucleotide and an amino acid were taken as 350 and 100, respectively.

The molecular weight of deoxynucleotide kinase synthesized with T4 8-min RNA, 15 S, or 12 S RNA in vitro (average value, 48,000) is in agreement with that of the enzyme synthesized in vivo, 52,000. We have also indicated that purified deoxynucleotide kinase is composed of two identical subunits, the molecular weight of each subunit being about 23,000. We believe that these subunits are identical since they did not separate upon electrophoresis on polyacrylamide gel columns in the presence of SDS. Analysis of the number and kind of NH2-terminal amino acids would be required to establish this point conclusively.

Although recognizing the hazards of estimating molecular weights of RNA samples from sedimentation coefficients obtained from centrifugation on sucrose density gradients (12), we feel that the agreement between the molecular weight of the kinase subunit (23,000) and that of a protein translated from 12 S RNA (27,000) is sufficiently good to propose that one species of 12 S RNA is not only the product of the transcription of a single gene of T4 DNA but contains all the required information to permit its translation into a biologically active kinase subunit. The 15 S species contains enough information for the kinase subunit plus probably one other protein.

The production of two distinct species of kinase mRNA in vivo might result from the manner in which T4 DNA is transcribed or from a specific cleavage of 15 S RNA. We have shown in unpublished experiments that the kinase messenger of 15 S and 12 S RNA occurs in approximately the same ratio, regardless of the time of extraction of RNA after T4 infection, or whether chloramphenicol was added at 3 min after infection. Thus, there appears to be no obvious precursor-product relationship between 15 S and 12 S RNA.

For this reason we believe that the ratio of 15 S and 12 S kinase mRNA found in vivo represents the physiological pattern of transcription of T4 DNA. In this specific instance, the kinase mRNA found in 12 S does not arise by elongation of immediate-early strands as has been suggested for the synthesis of some delayed early mRNAs in vitro (15-17) and in vivo (18). Initiation and termination of the synthesis of this kinase mRNA occurs within the gene 1 cistron of T4 DNA.

We have considered two possible mechanisms for the transcription of the kinase gene to obtain 15 S and 12 S messengers. In the first case the promoter site is on the kinase gene and transcription continues throughout the adjacent gene to give a 15 S RNA or stops at the end of gene 1 to yield the 12 S fragment. In the second case, a promoter site is present in the adjacent gene, but transcription occurs over both cistrons of which the kinase gene is promoter distal. A weaker promoter site on gene 1 permits the formation of some monocistronic kinase mRNA. Sederoff et al. (19) and Schmidt et al. (20) have described experiments of a similar nature, which show that the rIIa RNA, a delayed-early species, may be found both as a monocistronic or polycistronic unit. The kinetics of appearance of rIIa and rIIb messengers demonstrate that transcription can be initiated on...
its position or conformation on the bacterial membrane. Several models of transcription of the "early" T4 genes have been proposed to account for the observation that delayed-early RNA is not formed in vivo when cells are infected in the presence of chloramphenicol. This observation has been interpreted as evidence for active control of delayed-early transcription mediated by either phage-specific initiation factor(s) (21) or anti-termination (i.e. anti-p) factors (22). The apparent lack of requirement for specific phage protein for transcription of some delayed-early species may be related to the formation of short segments of DNA as described for transcription of late genes, but, instead, might be related to some other property of DNA, for example, its position or conformation on the bacterial membrane.

This model would stipulate that a phage-specific protein for transcription of gene 1 protein is needed in vivo but not necessarily in vitro, if, in the course of DNA extraction and preparation, the kinase promoter site becomes available for binding to RNA polymerase. The conclusion reached for the transcription of gene 1 need not exclude previous passive models for other delayed-early genes. They do indicate, however, that special conditions may be associated with the formation of this particular gene product.

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