Binding of Escherichia coli RNA Polymerase to T7 DNA

DISPLACEMENT OF HOLOENZYME FROM PROMOTER COMPLEXES BY HEPARIN*

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Escherichia coli RNA polymerase holoenzyme bound to promoter sites on T7 DNA is attacked and inactivated by the polyanion heparin. The highly stable RNA polymerase-T7 DNA complex formed at the major T7 Al promoter can be completely inactivated by treatment with heparin, as shown by monitoring the loss of activity of such complexes, and by gel electrophoresis of the RNA products transcribed. The rate of this inactivation is much faster than the rate of dissociation of RNA polymerase from promoter complexes, and thus represents a direct attack of heparin on the polymerase molecule bound at promoter A1. Experiments employing the nitrocellulose filter binding technique suggest that heparin inactivates E. coli RNA polymerase when bound to T7 DNA by directly displacing the enzyme from the DNA. RNA polymerase bound at a minor T7 promoter (promoter C) is much less sensitive to heparin attack than enzyme bound at promoter A1. Thus, the rate of inactivation of RNA polymerase-T7 DNA complexes by heparin is dependent upon the structure of the promoter involved even though the inhibitor binds to a site on the enzyme molecule.

Heparin has been shown to be an effective inhibitor of bacterial and eukaryotic DNA dependent RNA polymerases (1-3). Heparin is a highly sulfated linear polysaccharide (4). This polyanion inactivates free RNA polymerase at a faster rate than RNA polymerase which is bound to DNA (1). Sedimentation studies (1) and filter binding experiments (2) suggest that heparin inhibits RNA polymerase by forming a complex with the enzyme, possibly at the site involved in binding of the DNA template. The polyanionic character of the heparin molecule appears to be responsible for this binding; enzymes such as ribonuclease, which resemble RNA polymerase in their request for a substrate bearing regularly repeating negative charges, are also inhibited by heparin. In addition, a number of other polyanions are also inhibitors of RNA polymerases including polyethanesulfonate (5), aurintricarboxylic acid (6), and various polynucleotides such as poly(rU), poly(rU), and RNA (7-12).

Binary complexes of RNA polymerase bound to T7 DNA formed at low temperatures or elevated ionic strength are much more sensitive to attack by heparin than are the corresponding complexes formed at elevated temperatures and low ionic strength (2, 12). For this reason many authors have used heparin to study the properties of promoter complexes (13-16). In particular it is frequently assumed that "nonspecific" complexes formed by RNA polymerase at randomly encountered sites on the DNA template are attacked by heparin, while "specific" complexes formed at promoter sites are not. We were led to explore this matter in more detail by the observation that complexes formed by Escherichia coli RNA polymerase holoenzyme at T7 promoters could be rapidly inactivated by heparin (14, 17).

When T7 DNA is employed as template, E. coli RNA polymerase catalyzes the synthesis of RNA which is predominantly of a single class size (2 to 2.4 x 10^5; (18)) and which is initiated at three promoter sites (A1, A2, A3) clustered near the left end of the genome (19, 20). When RNA polymerase is added to T7 DNA (in the absence of nucleoside triphosphates) at a concentration in excess of that needed to saturate these A promoter sites, it binds tightly at several additional promoter sites designated T7 promoters B, C, D, and E (17). These have been designated "minor" promoter sites since E. coli RNA polymerase preferentially uses the A sites when given a choice. Enzyme bound at these minor promoters forms highly stable complexes which closely resemble those formed at A1, A2, and A3 in terms of being able to initiate RNA chains rapidly (12, 17). (These are "open" promoter complexes in terms of current models for transcription (13.) However, the rate of formation of complexes at these minor promoters is reduced as compared to the A promoter sites (17). The RNA products from all of these promoters can easily be separated and identified by gel electrophoresis, which provides a direct method for comparing the susceptibility of several T7 promoter complexes to inactivation by agents such as heparin.

EXPERIMENTAL PROCEDURES

Materials – RNA polymerase was purified from an RNase I-deficient strain (DG156) of Escherichia coli according to the procedure of Burgess and Jendrisak (21). Fraction D from this procedure was then chromatographed on phosphocellulose in the presence of 50% glycerol to isolate the RNA polymerase holoenzyme (22). This enzyme fraction contained a full equivalent of sigma subunit as determined by denaturation scanning of polyacrylamide gels and as determined using a rifampicin challenge assay procedure (23). The specific activity of the polymerase was 20,000 units per mg of protein where 1 unit is defined as 1 nmoI of acid-insoluble CMP incorporated in 1 h with T7 DNA as template. About 65% of the enzyme molecules were able to initiate an RNA chain as determined by 32P-labeled nucleotide incorporation.1 References in the text to "active" molecules corre-

1 W. Nierman, personal communication.
spond to this latter value. Protein concentrations were determined using the method of Lowry et al. (24) after precipitation of the sample with 3.5% trichloroacetic acid. Bovine serum albumin was used as a standard. The true concentration of RNA polymerase protein in amino acids is 0.8 times the concentration of protein determined by the Lowry method (12), and it is this corrected concentration that is given in all experiments. All enzyme to DNA ratios are calculated from the value for active RNA polymerase molecules.

DNA was isolated from purified phage by phenol extraction as described by Thomas and Abelson (25). The T7 deletion mutant AD111 was provided by F. W. Studier, Brookhaven National Laboratory. 32P-labeled inorganic phosphate was purchased from New England Nuclear Corp. and was converted to [α-32P]CTP by the method of Symons (26). [14C]ATP was purchased from Schwarz-Mann and unlabeled nucleoside triphosphates were purchased from Cal-Biochem and P-L Biochemicals. Bovine serum albumin was purchased from Miles and was acetylated by the method of Dowhan (27) to inactivate any contaminating nucleases.

A purified sample of heparin (Roche Laboratories) was kindly provided by W. Zillig (2), and was used except where noted. Other heparin preparations were purchased from Schwarz/Mann and Sigma, as the sodium salts. Heparin solutions were assayed for endonucleolytic DNase activity following the procedure of Kühnein et al. (28) with minor modifications. Reaction mixtures of 0.15 ml, identical to those used to assay RNA synthesis, were incubated for 8 min at 37°C. Reactions were stopped with 1.0 ml of 0.01% sodium dodecyl sulfate, 2.5 × 10−3 M EDTA, pH 7.0, and the octanol extraction step was omitted. Ribonuclease activity was assayed successfully by monitoring the reduction in size of T7 transcripts I and II formed by T7 RNA polymerase.

**RNA Polymerase Assays** —RNA polymerase was assayed essentially as described by Berg et al. (29) with the following modifications. Enzyme (stored at −20°C in 50% glycerol) was diluted with a solution containing 10 mM Tris/Cl pH 8.0, 10 mM MgCl2, 10 mM β-mercaptoethanol, 50 mM KCl, 0.1% sodium dodecyl sulfate, 25 μg/ml of acetylated bovine serum albumin, and 5% glycerol. Assay mixtures of 0.1 ml contained 40 mM Tris/Cl pH 8.0, 10 mM MgCl2, 10 mM β-mercaptoethanol, and 0.1 mg/ml of acetylated bovine serum albumin. Binary complexes of RNA polymerase bound to DNA were formed as described by Mangel and Chamberlin (25). [14C]ATP (2 to 10 εpmol) or [α-32P]CTP (5 to 125 εpmol) were used as the labeled nucleotides. RNA synthesis was started by the addition of nucleoside triphosphates in a total volume of 0.1 ml. After 6 min at 37°C, synthesis reactions were stopped with 2.5 ml of ice-cold 3.5% perchloric acid containing 0.1 M NaF.

**Methods** —RNA synthesis reactions for gel electrophoresis (final volume of 0.1 ml) were stopped by the addition of 25 μl of a solution containing 0.1 M EDTA, 0.1% sodium dodecyl sulfate, 27 mM Tris/Cl, 27 mM boric acid, 0.1% bromophenol blue dye, and 50% glycerol. A 10-μl sample was then assayed for incorporation of CMP into acid-insoluble material. An appropriate portion of the reaction solution was then analyzed by electrophoresis on 2% acrylamide and 0.5% agarose (30) slab gels as described previously (31). Nitrocellulose filter binding experiments were carried out as described by Hinkle and Chamberlin (19) except that the binding buffer was identical with the enzyme diluent described above. Heparin attack conditions are described in each experiment; all experiments were carried out at 37°C.

**RESULTS**

**Heparin Inactivation of RNA polymerase-T7 DNA Complexes** —AD111 DNA is a T7 deletion mutant which lacks two of the three strong A promoters at the left end of the genome (32). When RNA polymerase is allowed to bind to this DNA at a low enzyme to DNA ratio, transcription occurs primarily from T7 promoter A1 (17). Synthesis of RNA by these DNA complexes therefore directly reflects the properties of the enzyme when bound to this specific promoter site. To test the effect of heparin on RNA polymerase complexes at the A1 promoter, stable complexes were formed by mixing RNA polymerase with D111 DNA for 8 min at 37°C (23). Heparin was then added and incubation was continued for various times. The reaction was stopped by the addition of a mixture of four ribonucleoside triphosphates; in the presence of triphosphates any effective promoter complexes immediately initiate and begin elongation of an RNA chain (14, 23). The elongation of RNA chains is not blocked by heparin. Under these conditions incorporation of labeled nucleotides into RNA directly reflects the fraction of active RNA polymerase-DNA complexes present. In the presence of increasing amounts of heparin the polymerase-promoter complexes are progressively inactivated (Fig. 1, left). This inactivation is much faster than the rate of dissociation of RNA polymerase from promoter complexes, which requires many hours under these conditions (12, 33, 34), and thus must represent a direct attack of heparin on the polymerase molecule bound at the A1 promoter site.

To establish that heparin was indeed attacking RNA polymerase when bound to the major A1 promoter, polycrylamide gel electrophoresis of the RNA products synthesized under these conditions was carried out (Fig. 2, left). After the addition of substrates, RNA chains were allowed to grow to their full lengths, incorporating [32P]CMP as the labeled nucleotide. In the absence of heparin (Track a), essentially all of the labeled nucleotide is found in a band with the electrophoretic mobility characteristic of T7 RNA A1 (17), although traces of minor transcripts from promoters C and E are detected. When heparin is added at a final concentration of 10 μg/ml and samples are allowed to begin RNA synthesis after increasing periods of exposure, species A1 RNA clearly begins to disappear after 90 min (Track c) and is barely present after 100 min (Track e). This confirms our conclusion that heparin is able to directly attack Escherichia coli RNA polymerase bound in highly stable complexes at the major T7 A1 promoter.

It is interesting that the small amount of species C RNA formed in these experiments is not lost even after 100 min in the presence of this concentration of heparin. This transcription from the C promoter is probably responsible for the small fraction of "heparin-resistant" transcript seen at later times in Fig. 1 (see below).

Although over 90% of the RNA polymerase complexes are formed at promoter A1, the kinetic form of the inactivation
reaction is complex. If the approximate molecular weight of heparin is 12,000 (1, 4), then 10 μg/ml represents a large (200-fold) molar excess of heparin over the concentration of active RNA polymerase. If the reaction we observe has the form:

$E + DNA + heparin \rightarrow E.heparin + DNA$

then pseudo-first order kinetics might be expected from the simplest reaction mechanism. In fact, the graph of ln (fractional activity) as a function of time does not give a simple linear relationship, from which we conclude that the reaction is not a simple second order process. There may well be several steps involved in the displacement. Alternatively we cannot rule out the possibility that there are several forms of heparin, perhaps varying in their density of negative charge, which differ significantly in their intrinsic rates of attack on RNA polymerase complexes.

**Inactivation of RNA polymerase at Other T7 Promoters by Heparin**—When RNA polymerase is allowed to bind to T7 ΔD111 DNA at a high enzyme to DNA ratio, the enzyme forms highly stable complexes at the T7 minor promoters C, D, and E in addition to promoter A1 (17). When kinetic experiments carried out under these conditions are analyzed by gel electrophoresis one obtains a direct comparison of the sensitivity of complexes at these four T7 promoters to attack by heparin. Experiments were carried out at an enzyme to DNA ratio of 5, using the experimental protocol described above. Heparin was added to final concentrations of 0.01, 0.1, and 1.0 mg/ml and the ability of complexes to incorporate labeled nucleotides into RNA was assayed after increasing periods of heparin exposure (Fig. 1, right). The kinetic form of these reactions is different than that seen at left in Fig. 1. The curves reach a slowly decaying plateau, and the complexes require substantially higher concentrations of heparin for inactivation. We attribute these differences to the fact that RNA polymerase holoenzyme bound at promoter C is far more resistant to attack than that bound at promoter A1. This was suggested in the first experiment; however, under the conditions of Fig. 1, right, a substantial portion of the enzyme is bound at this promoter. This hypothesis was verified by electrophoretic analysis of the RNA products synthesized in this experiment (Fig. 2, right). At a heparin concentration of 0.2 mg/ml, species A1 RNA completely disappears after complexes have been treated for 10 min (Track b), and species C RNA just begins to decrease in intensity after 90 min (Track d). As shown previously (17), RNA polymerase bound at promoter E is most sensitive to attack by heparin. Thus the inactivation of RNA polymerase-T7 DNA complexes by heparin varies considerably for these three different promoter sites and, interestingly, shows no direct correlation with the "strength" of the promoter.

An experiment identical to Fig. 2, right, was also carried out using wild type T7 DNA at an enzyme concentration sufficient to saturate the three A promoter sites (data not shown). Under these conditions, enzyme bound at sites giving rise to species A RNA was attacked by heparin at a rate comparable to that found for ΔD111 in Fig. 2, left. We conclude that binary complexes formed at promoters A2 and A3 are at least as sensitive to heparin attack as promoter A1, although we have not measured the rate of attack at the individual A promoter sites.

**Displacement of RNA Polymerase from Promoter Complexes by Heparin**—We have shown above that heparin inactivates RNA polymerase when bound to T7 promoters in a highly stable complex. There are several plausible reaction mechanisms for this inactivation; one mechanism involves the physical displacement of the enzyme from the DNA. To test this possibility, nitrocellulose filter binding experiments were carried out according to the procedure of Hinkle and Chamberlin (12). This technique involves mixing RNA polymerase with a radioactively labeled DNA followed by filtration through a nitrocellulose filter. RNA polymerase-DNA complexes are retained on the filter (9, 12) and can be quantitatively determined by measurement of the radioactivity bound. If heparin displaced the enzyme from the complex, the DNA will not be retained, except for a low (2 to 3%) background level. The experiment was carried out as follows: complexes of RNA polymerase bound to T7 [3H]DNA were formed at an enzyme concentration sufficient to saturate the three A promoter sites (data not shown). Under these conditions, enzyme bound at sites giving rise to species A RNA was attacked by heparin at a rate comparable to that found for ΔD111 in Fig. 2, left. We conclude that binary complexes formed at promoters A2 and A3 are at least as sensitive to heparin attack as promoter A1, although we have not measured the rate of attack at the individual A promoter sites.

**Fig. 2.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Left, the kinetics of inactivation of RNA polymerase-T7 ΔD111 complexes at promoter A1 by heparin. The conditions are identical with Fig. 1 at 0.01 mg/ml of heparin, except that [α-32P]CTP (140 cpm/nmol) was used as the labeled nucleotide. Equal amounts of labeled RNA from these reactions were analyzed per track. Track a, no heparin; Track b, 10 min; Track c, 30 min; Track d, 60 min; and Track e, 100 min. The amount of CMP incorporated in each of these reactions was a, 0.46 nmol; b, 0.21 nmol; c, 0.1 nmol; d, 0.08 nmol; and e, 0.05 nmol. Right, the kinetics of inactivation of RNA polymerase at ΔD111 DNA promoters A1, C, and E by heparin. The heparin concentration was 0.2 mg/ml. Equal amounts of [32P]CMP-labeled RNA from these reactions were analyzed per track. Track a, no heparin; Track b, 10 min; Track c, 30 min; Track d, 90 min. The amount of CMP incorporated in each of the reactions is a, 1.2 nmol; b, 0.55 nmol; c, 0.30 nmol; d, 0.3 nmol.
polymerase binds primarily to one of the three A promoters at total 3H-labeled T7 DNA added. The kinetics of heparin disruption of RNA polymerase T7 DNA complexes. RNA polymerase (0.06 μg) was incubated with 5 nmol of 3H-labeled T7 DNA (3000 cpm/nmol) in a binding reaction mixture described under "Experimental Procedures." This mixture was incubated at 37° for 8 min, when heparin was added as described in the text. The value for 100% complexes bound was determined by the addition of heparin immediately prior to filtration, and represents 40% of the total 3H-labeled T7 DNA added. Δ——Δ = 0 mg/ml of heparin, O——O = 0.01 mg/ml of heparin. Middle, kinetics of heparin disruption of all RNA polymerase T7 DNA complexes. RNA polymerase (0.24 μg) was incubated as described above, and the final heparin concentration was 1 mg/ml. The value for 100% complexes bound as determined above represents 35% of total 3H-labeled T7 DNA added. In the absence of heparin, 55% of the total 3H-labeled T7 DNA was bound to the filter. Bottom, kinetics of heparin disruption of all RNA polymerase T7 DNA complexes. The final heparin concentration was 0.01 mg/ml. The value for 100% complexes bound as determined above represents 55% of the total 3H-labeled T7 DNA added.

similar experiments were carried out at an enzyme to DNA ratio of about 5.2, at a heparin concentration of 1 mg/ml (Fig. 3, middle). This concentration of heparin inactivates all the complexes present under these conditions with a half-time of about 6 min (Fig. 1, right). The corresponding half-time for disruption of the complex as measured by the nitrocellulose filter binding technique (see Fig. 3, middle) is about 7 min. Because the rate of heparin inactivation of enzyme-DNA complexes and the rate of heparin displacement of enzyme from the DNA are virtually identical, we conclude that heparin inactivates RNA polymerase-T7 DNA complexes by directly displacing the enzyme from the DNA. It cannot be ruled out, of course, that heparin simply adds to the binary complex and that this ternary complex is not retained on the nitrocellulose filter. We consider this unlikely, since a very large number of protein-ligand complexes are retained on the filters, and we can see no reason why the presence of heparin bound to RNA polymerase should dramatically alter its ability to be retained.

Under conditions where RNA polymerase is bound to several promoter sites per DNA molecule, one predicts that at an intermediate heparin concentration, some of the binary complexes will not be disrupted. For example, complexes formed at promoter C should not be disrupted after short periods of exposure, whereas complexes at promoter A1 should be displaced (see above). This prediction was tested in the following manner: RNA polymerase was mixed with T7 [3H]DNA at an enzyme to DNA ratio of about 5.2, as described above. The disruption of complexes was examined by nitrocellulose filter binding at a heparin concentration of 0.01 mg/ml (Fig. 3, bottom). As expected, we observed a slight decrease in [3H]DNA bound to the filter, which presumably represents the decrease in efficiency of binding due to the displacement of some but not all of the enzyme molecule bound. However, the major fraction of DNA remains nonfilterable; that is, the more resistant complexes are stable to disruption by heparin, and these complexes do not lose their binding capacity.

**DISCUSSION**

Heparin is useful as a selective inhibitor of free RNA polymerase and of certain nonspecific RNA polymerase-DNA complexes (1, 2, 15, 16). While it was previously believed that highly stable, open promoter complexes were resistant to attack by this inhibitor, we show here that complexes formed at all of the T7 promoters we have studied are attacked. However, the rate of attack on RNA polymerase bound at different promoter sites can vary over a considerable range. Surprisingly, the complexes formed at the major T7 A promoter site are considerably less stable than those formed at the minor promoter C. Under conditions of free site selection most E. coli RNA polymerase holoenzyme binds at the A promoter site. Thus heparin is not a reagent with which "strong" promoter complexes can be selectively isolated, unless one has previous knowledge of the heparin sensitivities of the promoters involved.

Several criteria were used to establish that the inhibitory activity of our heparin preparations was not due to a minor contaminant. First, our preparations contained no detectable RNase or DNase. Second, similar levels of inhibition were observed with heparin from several sources, and with heparin which had been fractionated by exclusion gel chromatography. Finally, spermidine, which would presumably interact with this polyanion, reduced the amount of inhibition substantially when present in the reaction mixtures (data not shown).
Why do different binary complexes differ in their sensitivity to heparin? One possibility we have considered is that different promoter sites have different affinities for RNA polymerase:

\[ E + \text{promoter} \rightleftharpoons K E - \text{promoter}. \]

Since RNA polymerase binds heparin tightly:

\[ E + \text{heparin} \rightleftharpoons E : \text{heparin} \]

then at equilibrium in the presence of an appropriate concentration of heparin, the inhibitor would selectively remove RNA polymerase from promoters with the lowest affinity for the enzyme. This would not be inconsistent with the order of heparin sensitivity we observe: we have shown elsewhere (12) that the major A promoters do not bind RNA polymerase more strongly than C and E; the selection of A as a major promoter appears to be due to differences in the rate of stable complex formation (17).

While this is a plausible explanation, it seems to us less likely than the notion that RNA polymerase bound at different promoters is attacked at different rates by heparin because the enzyme differs in its conformation in those different complexes. The time required to reach equilibrium by the first enzyme differs in its conformation in those different complexes. The time required to reach equilibrium by the first enzyme is altered by the structure of different promoter sites; these different conformations are attacked at quite different rates by inhibitors of the enzyme. This suggests the definite possibility that these different conformations might also respond selectively to different regulatory molecules.

REFERENCES

1. Walter, G., Zillig, W., Palm, P., and Fuchs, E. (1967) Eur. J. Biochem. 3, 194-201
2. Zillig, W., Zeche, K., Rabussay, D., Schaefer, M., Scharf, V. S., Palm, P., Heil, A., and Seifert, W. (1971) Cold Spring Harbor Symp. Quant. Biol. 35, 47-58
3. de Pomarai, D. I., Cheisterton, C. J., and Butterworth, P. H. W. (1974) FEBS Lett. 42, 149-153
4. Silva, M. E., and Dietrich, C. P. (1975) J. Biol. Chem. 250, 6841-6846
5. Chambron, P., Ramuz, M., Mandel, P., and Dolly, J. (1967) Biochim. Biophys. Acta 149, 584-596
6. Butenumal, T., and Landers, T. A. (1973) Biochim. Biophys. Res. Commun. 55, 680-688
7. Krakow, J., and Ochoa, S. (1963) Proc. Natl. Acad. Sci. U. S. A. 49, 88-94
8. Fox, C. F., Robinson, W. S., Hasselorn, R., and Weiss, S. B. (1964) J. Biol. Chem. 239, 186-195
9. Jones, O., and Berg, P. (1966) J. Mol. Biol. 22, 199-209
10. Richardson, J. (1966) J. Mol. Biol. 21, 83-113
11. Hirschbein, L., Dubert, J., and Babinet, C. (1967) Eur. J. Biochem. 1, 130-149
12. Hinkle, D., and Chamberlin, M. J. (1972) J. Mol. Biol. 70, 157-188
13. Chamberlin, M. J. (1974) Annu. Rev. Biochem. 43, 721-775
14. Chamberlin, M. J. (1976) in RNA Polymerase, Cold Spring Harbor Monograph Series (Losick, R., and Chamberlin, M., eds) pp. 151-191, Cold Spring Harbor Laboratory, New York
15. Schafer, R., Zillig, W., and Zechel, K. (1973) Eur. J. Biochem. 33, 207-214
16. Schafer, R., Kramer, R., Zillig, W., and Cudny, H. (1973) Eur. J. Biochem. 40, 267-273
17. Stahl, S. J., and Chamberlin, M. J. (1977) J. Mol. Biol. 112, 577-601
18. Millette, R. L., Tretter, C. D., Herrlich, P., and Schweiger, M. (1970) Cold Spring Harbor Symp. Quant. Biol. 35, 135-142
19. Minkley, E. G., and Prinhow, D. (1973) J. Mol. Biol. 77, 255-277
20. Dunn, J. J., and Studier, F. W. (1973) Proc. Natl. Acad. Sci. U. S. A. 71, 760-764
21. Burgess, R. R., and Jendrisak, J. J. (1975) Biochemistry 14, 4634-4638
22. Gonzalez, N., Wiggs, J., and Chamberlin, M. J. (1977) Arch. Biochem. Biophys. 182, 402-406
23. Rhodes, G., and Chamberlin, M. J. (1974) J. Biol. Chem. 250, 9112-9120
24. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
25. Thomas, C., and Abelson, J. (1966) in Procedures in Nucleic Acid Research (Cantoni, G. L., and Davies, D. R., eds) pp. 555-554, Harper and Row, New York
26. Symons, R. H. (1968) Biochim. Biophys. Acta 155, 609-610
27. Dowhan, W. (1969) Ph.D. thesis, University of California, Berkeley
28. Kuhnlein, U., Penhoet, E. E., and Linn, S. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1169-1173
29. Berg, D., Barrett, K., and Chamberlin, M. J. (1971) Methods Enzymol. 21, 506-519
30. DeWachter, R., and Fiers, W. (1973) Anal. Biochem. 49, 187-197
31. Golomb, M., and Chamberlin, M. (1974) J. Biol. Chem. 249, 2658-2663
32. Studier, F. W. (1975) J. Mol. Biol. 94, 283-295
33. Hinkle, D., and Chamberlin, M. (1976) Cold Spring Harbor Symp. Quant. Biol. 41, 85-97
34. Heyden, B., Nusslein, C., and Schaller, H. (1972) Nature New Biol. 240, 9-12
35. Wu, C. W., and Goldthwait, D. A. (1969) Biochemistry 8, 4454-4458
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