INHIBITION OF DNA-DEPENDENT RNA POLYMERASE OF E. COLI BY PHOSPHOLIPIDS*

Audrey Stevens
Biology Division, Oak Ridge National Laboratory,
Oak Ridge, Tennessee 37830

Received May 27,1975

SUMMARY: DNA-dependent RNA polymerase of E. coli is strongly inhibited by phosphatidylglycerol and cardiolipin at concentrations of 5 to 8 X 10^{-5} M. The two lipids inhibit the formation of rifampicin-resistant preinitiation complexes. Phosphatidylethanolamine is not inhibitory, and at a tenfold higher concentration partially overcomes the inhibition by the other two lipids.

Several lines of evidence have suggested that DNA-dependent RNA polymerase of E. coli has hydrophobic sites. For example, a lipophilic class C rifamycin derivative, AF/013, inhibits RNA polymerase, and many molecules of the inhibitor bind to the enzyme (1). Also, Ishihama et al. (2) have reported that RNA polymerase which has lost activity is greatly activated by brief exposure to alkali. Such exposure previously had been shown to increase the activity of microsomal enzymes because of its ability to alter lipid-protein interactions (3, 4). I have observed that nonionic detergents replace bovine serum albumin in maintaining the stability of RNA polymerase in reaction mixtures; this finding led me to investigate the effect of the major lipids (phospholipids) of E. coli on the activity of the enzyme, as reported in this paper.

MATERIALS AND METHODS

RNA polymerase holoenzyme, core enzyme, and sigma were prepared as previously described (5).

Assay mixtures contained [^{14}C]ATP (0.25 mM, 1500 cpm/nmole; Schwarz/Mann); UTP, CTP, and GTP (0.25 mM each; P-L Biochemicals, Inc.); Tris buffer (20 mM, pH 7.8); $MgCl_2$ (10 mM); 2-mercaptoethanol (10 mM); T4 DNA (10 µg, isolated from T4D

*This investigation was supported by the Energy Research and Development Administration under contract with the Union Carbide Corporation.

phage according to the procedure of Thomas and Abelson, ref. 6) or calf thymus DNA (20 μg; Worthington Biochemicals Corp.); bovine serum albumin (100 μg; Schwarz/Mann); and enzyme. Incubation was for 10 min at 37°, and determination of radioactivity incorporated

TABLE 1

Effect of Phospholipids on the Activity of RNA Polymerase*

Phospholipid	Concentration (M)	Enzyme Fraction	DNA Template	Inhibition (%)
Experiment 1				
Phosphatidyl-ethanolamine	7.5×10^{-4}	core enzyme + sigma	T4	0
Lysophosphatidyl-ethanolamine	6×10^{-4}	core enzyme + sigma	T4	0
Lecithin	1.5×10^{-3}	core enzyme + sigma	T4	0
Phosphatidyl-glycerol	1.5×10^{-5}	core enzyme + sigma	T4	17
	3.2×10^{-5}			38
	8×10^{-5}			69
	1.6×10^{-4}			74
Cardiolipin	1×10^{-5}	core enzyme + sigma	T4	21
	2×10^{-5}			45
	5×10^{-5}			70
	1×10^{-4}			75
Experiment 2				
Cardiolipin	3×10^{-5}	core enzyme + sigma	T4	52
	6×10^{-5}	core enzyme + sigma	calf thymus	45
	6×10^{-5}	core enzyme	T4	38
	6×10^{-5}	core enzyme	calf thymus	11
	6×10^{-5}	holoenzyme	T4	42

*The reaction mixtures were as described in Materials and Methods. Core enzyme was present at a concentration of 2 μg, sigma subunit at a concentration of 0.2 μg, and holoenzyme at a concentration of 3 μg.

into RNA was as previously described (7). Lipids were added last to reaction mixtures at 0°.

Rifampicin was obtained from Calbiochem and phospholipids from Supelco, Inc., Bellefonte, Pa. Micelle preparations were prepared by sonication for 90 sec at 0° using 10 mM Tris buffer (pH 7.8), 5 mM EDTA, 10 mM dithiothreitol as a suspending buffer. Phospholipids were determined by phosphate analyses according to Ames (8).

RESULTS

The effect of phospholipids on the activity of RNA polymerase core enzyme plus sigma was first studied. The phospholipids tested were phosphatidylethanolamine, phosphatidylglycerol, cardiolipin, lecithin, and lysophosphatidylethanolamine. Phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin are the three principal lipids of E. coli, being found

TABLE 2

Effect of Phosphatidylglycerol and Cardiolipin on Rifampicin-Resistant DNA-Enzyme Preinitiation Complex Formation*

Experiment No.	Phospholipid Added	[^{14}C]AMP Incorporated (nmoles)
1. Lipid added with enzyme and DNA before preincubation period	none	0.17
	phosphatidylglycerol 8×10^{-5} M	0.04
	cardiolipin, 5×10^{-5} M	0.03
2. Lipid added with ribonucleoside triphosphates and rifampicin after preincubation period	none	0.18
	phosphatidylglycerol 8×10^{-5} M	0.19
	cardiolipin, 5×10^{-5} M	0.16

*Reaction mixtures for preinitiation complex formation contained Tris buffer, $MgCl_2$, mercaptoethanol, T4 DNA, and bovine serum albumin as described in Materials and Methods. Core enzyme and sigma subunit were present at concentrations of 2 μg and 0.2 μg respectively. The mixtures were preincubated for 5 min at 37°. Rifampicin (2 μg) and ribonucleoside triphosphates were then added and the mixtures incubated for an additional 10 min at 37°.

in the ratio 10:3:1, respectively (9). Phosphatidylglycerol and cardiolipin at low concentrations were found to be inhibitory to RNA polymerase, but the other phospholipids had no effect on the activity. Table 1, Experiment 1, shows the results. Cardiolipin and phosphatidylglycerol inhibit strongly at 5×10^{-5} M and 8×10^{-5} M, respectively. Holoenzyme and core enzyme alone were inhibited less than core enzyme plus sigma (Table 1, Experiment 2). Core enzyme plus sigma with T4 DNA as a template showed the greatest inhibition.

Rifampicin inhibits RNA synthesis by binding to the polymerase. If RNA polymerase–DNA complexes are first formed by incubating reaction mixtures in the absence of ribonucleoside triphosphates, the enzyme is highly protected from rifampicin (10). Thus, rifampicin-resistant RNA polymerase-DNA complexes are a measure of the amount of preinitiation complex formation which has occurred. To determine the stage of synthesis at which the two phospholipids are inhibitory to the enzyme, the number of rifampicin-resistant RNA polymerase–DNA complexes formed during a 5-min preincubation period

TABLE 3

Phosphatidylethanolamine Relief of the Inhibition by Phosphatidylglycerol and Cardiolipin*

Addition	[^{14}C]AMP Incorporated (nmoles)
None	0.52
Phosphatidylglycerol (3×10^{-5} M)	0.38
Phosphatidylethanolamine (3×10^{-4} M)	0.57
Both of above	0.49
None	0.58
Cardiolipin (3×10^{-5} M)	0.25
Phosphatidylethanolamine (3×10^{-4} M)	0.55
Both of above	0.38

*Reaction mixtures were as described in Materials and Methods. Core enzyme was present at a concentration of 2 µg and sigma subunit at a concentration of 0.2 µg. T4 DNA was used as a template.

was measured in the absence and presence of the phospholipids. Phospholipids were also added to reaction mixtures along with rifampicin and ribonucleoside triphosphates after preinitiation complex formation. As shown in Table 2, the phospholipids were very inhibitory to preinitiation complex formation, but were not inhibitory when added along with the ribonucleoside triphosphates and rifampicin.

Since phosphatidylethanolamine is not inhibitory to the enzyme, it was tested to see whether it could overcome the inhibition by the other two phospholipids of E. coli. The results are shown in Table 3. At a concentration 10 times higher than that of the inhibitory lipid, phosphatidylethanolamine relieves the inhibition of phosphatidylglycerol by 65% and that of cardiolipin by about 40%.

The results suggest the possibility that the activity of RNA polymerase is affected by the lipid environment of the bacterial membrane fraction. Menon (11) has reported previously on a possible role of lipids with RNA polymerase from mammalian cells. Using a particulate enzyme fraction from rat liver nuclei, he found that pretreatment of the enzyme with phospholipase A or phospholipase C decreased its activity, as did extraction of the enzyme with ether or iso-octane.

REFERENCES

1. Riva, S., Fietta, A., and Silvestri, L. G., Biochem. Biophys. Res. Commun. 49, 1263-1271 (1972).
2. Ishihama, A., Fukuda, R., and Ito, K., J. Mol. Biol. 79, 127-136 (1973).
3. Stetten, M. R., and Burnett, F. F., Biochim. Biophys. Acta 128, 344-350 (1966).
4. Vessey, D. A., and Zakim, D., J. Biol. Chem. 246, 4649-4656 (1971).
5. Stevens, A., Biochemistry 13, 493-503 (1974).
6. Thomas, C. A., Jr., and Abelson, J., Procedures in Nucleic Acid Research (G. L. Cantoni and D. K. Davies, eds.), pp. 553-561. New York, Harper and Row, 1966.
7. Stevens, A., and Henry, J., J. Biol. Chem. 239, 196-203 (1964).
8. Ames, B. N., Methods Enzymol. 8, 115-118 (1966).
9. Ames, G. F., J. Bacteriol. 95, 833-843 (1968).
10. Hinkle, D. C., Mangel, W. F., and Chamberlin, M. J., J. Mol. Biol. 70, 209-220 (1972).
11. Menon, I. A., Can. J. Biochem. 50, 807-812 (1972).