Polyamines in the Synthesis of Bacteriophage Deoxyribonucleic Acid

I. Lack of Dependence of Polyamine Synthesis on Bacteriophage Deoxyribonucleic Acid Synthesis

ARNOLD S. DION AND SEYMOUR S. COHEN¹

Department of Therapeutic Research, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Received for publication 9 November 1971

To determine whether polyamine synthesis is dependent on deoxyribonucleic acid (DNA) synthesis, polyamine levels were estimated after infection of bacterial cells with ultraviolet-irradiated T4 or T4 *am* N 122, a DNA-negative mutant. Although phage DNA accumulation was restricted to various degrees in comparison to cells infected with T4D, nearly commensurate levels of putrescine and spermidine synthesis were observed after infection, regardless of the rate of phage DNA synthesis. We conclude from these data that polyamine synthesis after infection is independent of phage DNA synthesis.

It has become increasingly evident that polyamines perform an important function in the biosynthesis and accumulation of ribonucleic acid (RNA; reference 4). The possible function(s) of polyamines in deoxyribonucleic acid (DNA) synthesis and metabolism has not been extensively investigated; however, bacteria infected by T-even phage offer a unique system for the study of polyamine involvement in DNA synthesis, without the concomitant accumulation of RNA.

Hershey (11) first reported the presence of two low-molecular-weight, ninhydrin-positive compounds, which were derived from arginine, in purified T-even phage preparations. Not only were these compounds shown to be injected into the bacterial host with the phage DNA, but it was also shown that similar compounds were present in the uninfected host. Ames and Dubin (2) later identified these compounds as putrescine and spermidine and estimated that approximately 40 to 50% of the phage DNA phosphate could be neutralized by these organic cations.

Cohen and Raina (8) have demonstrated a considerable net synthesis of putrescine and spermidine after infection, paralleling DNA accumulation. The present investigation is concerned with the possible dependence of polyamine synthesis on the synthesis of phage DNA. We have previously reported that DNA synthesis and phage

morphogenesis are markedly stimulated by exogenous polyamines in an infected host which is inhibited in endogenous polyamine synthesis (6) and have extended these observations in the accompanying paper (9).

MATERIALS AND METHODS

Preparation of phage stocks. We have used r^+ strains under conditions of multiple infection to minimize lysis and to prolong DNA synthesis. T4 *am* N 122 (gene 42) defective in deoxycytidylate hydroxymethylase activity was obtained from W. B. Wood (California Institute of Technology, Pasadena, Calif.). Phage lysates of T4 and T4 *am* N 122 were prepared by infecting *Escherichia coli* B and *E. coli* CR 63, respectively, at a multiplicity of infection (MOI) of 0.1 and incubating overnight. Purified phage stocks were obtained by two cycles of low ($4,080 \times g$) and high ($27,300 \times g$)-speed centrifugation of broth lysates. Ultraviolet-irradiated stocks of T4D were prepared by irradiating stirred phage stocks with a G.E. germicidal lamp. The number of lethal hits per phage was estimated from per cent survival, employing the Poisson distribution. Plaque-forming units were assayed by the soft agar overlay method as described by Adams (1).

Phage infections. An overnight culture of *E. coli* B in medium 52 (5) was diluted with the same medium containing glucose (3 mg/ml) to a cell density of approximately 5×10^7 /ml. These cells were grown at 37 C to a cell density of approximately 2×10^8 /ml. After addition of L-tryptophan (final concentration of 25 μ g/ml), a concentrated purified virus preparation was added (MOI = 5), and the culture was mixed immediately.

¹ Present address: Department of Microbiology, University of Colorado, School of Medicine, Denver, Colo. 80220.

FIG. 1. Spermidine and DNA synthesis in *E. coli B* infected with T4D and UV-irradiated T4D. Exponentially growing cultures of *E. coli B* cells at an approximate density of 2×10^8 /ml [turbidity (*T*) in Klett (420 filter) = 80] were infected at an MOI of 5 with unirradiated T4D (A) and T4D containing 3.4 (B) and 6.5 (C) lethal hits per phage. At the indicated times, samples were removed for DNA and polyamine analyses. (A-C.) Spermidine levels in cells (Δ, total; ○, free) and cells plus medium (×, total; ●, free). (D) DNA: □, unirradiated; ○, 3.4 lethal hits/phage; ▲, 6.5 lethal hits/phage.

Polyamine and DNA analyses. The polyamines in acid extracts were assayed as fluorescent dansyl derivatives (10). The compounds were separated by thin-layer chromatography on activated silica gel plates (Analtech, Newark, Del.), with ethyl acetate-cyclohexane (30:60; v/v), and the fluorescent spots were scanned on the Turner fluorimeter. For separate determinations of polyamine content of cells and media, membrane filters containing the filtered cells were extracted in 1.0 ml of 0.2 N perchloric acid (PCA). To the filtrate (medium) was added 2.0 N PCA to a final concentration of 0.2 N. Alternatively, the polyamine content of both cells and media was determined on cold 5% trichloroacetic acid extracts after centrifugation. Total polyamine, i.e., polyamine estimated after acid hydrolysis, was determined on PCA or trichloroacetic acid extracts by the addition of an equal volume of concentrated HCl and hydrolysis at 110°C for 18 hr. After the hydrolysate was taken to dryness over NaOH pellets and concentrated H_2SO_4 , the dry residue was resuspended in the original volume of 0.2

N PCA, and polyamines were estimated by dansylation as above. The diphenylamine reagent was used to assay DNA accumulation after infection (3).

RESULTS

The effects on DNA and spermidine synthesis of infecting *E. coli B* with UV-irradiated T4D are shown in Fig. 1. In comparison with cells infected with unirradiated T4D, the rates of phage DNA accumulation were inhibited approximately 28 and 47% in cells infected with irradiated phage suffering 3.4 and 6.5 lethal hits per phage, respectively. In addition, the initiation of DNA synthesis, especially in cells infected with the more heavily irradiated phage, was delayed. However, the net increase in spermidine content, i.e., in cells and media, was unaffected by either the delay in or the reduced rate of DNA accumulation. Although spermidine was present in both cells and media, no bound spermidine was observed, as evidenced by little or no increases in spermidine after HCl hydrolysis of cold acid extracts.

Similarly, as shown in Fig. 2, the net synthesis of putrescine after infection was unaffected by the inhibition or delay in the onset of DNA synthesis. Increases in the net synthesis of free putrescine

FIG. 2. Putrescine levels and turbidity changes in *E. coli B* infected with UV-irradiated T4D. Assays were performed on the same samples described in Fig. 1. (A-C) Putrescine levels in cells (Δ, total; ○, free) and cells plus medium (×, total; ●, free). (D) Turbidities in Klett units (420 nm): □, unirradiated; ○, 3.4 lethal hits/phage; ▲, 6.5 lethal hits/phage.

commenced at approximately 10 min after infection. From the start of infection, a bound form of putrescine soluble in cold acid accumulated almost exclusively in the medium. The nature of this bound form of putrescine has not been further investigated. The reduced levels of intracellular accumulation of spermidine and putrescine as demonstrated in Fig. 1C and 2C, respectively, are probably due to leakage resulting from a slower rate of cell wall repair. Evidence in favor of this idea is given in Fig. 2D; increases in the turbidity in cells infected with the more heavily irradiated phage were markedly inhibited.

The infection of a nonpermissive host with a DNA-negative (DO) mutant such as T4 *am* N 122 does not result in phage DNA accumulation and thus provides the ultimate test of whether polyamine synthesis is dependent on DNA synthesis. As shown in Fig. 3, DNA synthesis was almost totally inhibited after infection with this mutant; however, there was essentially no difference in spermidine synthesis in cells infected by T4D or T4 *am* N 122. In the latter infection, putrescine synthesis appeared to be slightly enhanced. As noted above, we again found little evidence for bound spermidine; however, rather large quantities of bound putrescine were clearly evident.

DISCUSSION

Although the syntheses of DNA and polyamines occur concurrently (8) and both of these newly synthesized substances are incorporated as nu-

cleates in the mature phage, the present study has demonstrated that the synthesis of polyamines is not controlled by phage DNA synthesis. This conclusion is similar to that reported by Cohen et al. (7) concerning the lack of dependence of net polyamine synthesis on RNA synthesis. Interestingly, there is little accumulation of any nucleic acid after infection of *E. coli* B by T4 *am* N 122; nevertheless, the course of polyamine synthesis is comparable to that observed in cells infected with T4D. In both systems, polyamine synthesis is characterized by a brief lag and linear production, suggesting that the inception of polyamine synthesis after interruption of normal growth by infection is not in fact triggered by DNA synthesis but depends upon quite independent factors.

These data combined with other data from this laboratory indicate that, although polyamines stimulate nucleic acid synthesis in phage infection, as will be seen in the accompanying paper (9), nucleic acid synthesis is not a prerequisite for polyamine synthesis in this system.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant 7005 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. Ames, B. N., and D. T. Dubin. 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. *J. Biol. Chem.* 235:769-775.
3. Cohen, S. S. 1948. The synthesis of bacterial viruses. I. The

FIG. 3. Polyamine levels and DNA synthesis in *E. coli* B infected with T4D or T4 *am* N 122. Exponentially growing cultures of *E. coli* B at an approximate cell density of 2×10^8 /ml ($T = 80$) were infected at an MOI of 5 with T4D or T4 *am* N 122. Samples for DNA and polyamine analyses were removed at the designated times; polyamine levels are expressed as concentration per milliliter of cells plus medium.

- synthesis of nucleic acid and protein in *Escherichia coli* B infected with T_{2r}⁺ bacteriophage. *J. Biol. Chem.* 174:281-293.
4. Cohen, S. S. 1971. Introduction to the polyamines. Prentice-Hall, Englewood Cliffs, N.J.
 5. Cohen, S. S., and R. Arbogast. 1950. Chemical studies in host-virus interactions. VII. A comparison of some properties of three mutant pairs of bacterial viruses, T_{2r}⁺ and T_{2r}, T_{4r}⁺ and T_{4r}, T_{6r}⁺ and T_{6r}. *J. Exp. Med.* 91:619-636.
 6. Cohen, S. S., and A. S. Dion. 1971. Polyamines and the multiplication of T-even phages, p. 266-287. Symposium on Nucleic Acid-Protein Interactions, Miami Winter Symposia 2, North-Holland, Amsterdam.
 7. Cohen, S. S., N. Hoffner, M. Jansen, M. Moore, and A. Raina. 1967. Polyamines, RNA synthesis, and streptomycin lethality in a relaxed mutant of *E. coli* strain 15 TAU. *Proc. Nat. Acad. Sci. U.S.A.* 57:721-728.
 8. Cohen, S. S., and A. Raina. 1967. Some interrelations of natural polyamines and nucleic acids in growing and virus-infected bacteria, p. 157-182. *In* H. J. Vogel, J. O. Lampen, and V. Bryson (ed.), *Organizational biosynthesis*, Academic Press Inc., New York.
 9. Dion, A. S., and S. S. Cohen. 1972. Polyamines in the synthesis of bacteriophage deoxyribonucleic acid. II. Requirement for polyamines in T4 infection of a polyamine auxotroph. *J. Virol.* 9:423-430.
 10. Dion, A. S., and E. J. Herbst. 1970. Polyamine changes during development of *Drosophila melanogaster*. *Ann. N.Y. Acad. Sci.* 171:723-734.
 11. Hershey, A. D. 1957. Some minor components of bacteriophage T₂ particles. *Virology* 4:237-264.