STABILIZATION OF BACTERIOPHAGE T5 BY SPERMINE AND RELATED POLYAMINES

Herbert Tabor

Laboratory of Pharmacology and Toxicology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

Received September 28, 1960

Bacteriophage T5 is markedly unstable when diluted in media of low ionic strength (Adams, 1949; Puck, 1949). Adams demonstrated that this instability was related to the divalent cation concentration, and could be markedly increased by the addition of sodium citrate or other chelating agents.

The recent findings (Mager, 1959; Tabor, 1960) that Escherichia coli protoplasts are stabilized by low concentrations of spermine and spermidine suggested the possibility that these polyamines would also stabilize bacteriophage T5. The experiments presented here demonstrate a protective effect with as little as 2×10^{-8} M spermine hydrochloride and 10^{-7} M spermidine hydrochloride.

Experimental

The titer of E. coli bacteriophage T5 decreased to < 2 per cent in less than 5 minutes when the phage was incubated at 37° in 0.001 M potassium phosphate buffer, pH 7.2, containing 2×10^{-3} M sodium citrate. (Tables 1 and 2.) However, in the presence of spermidine and spermine a marked degree of protection was observed, even at 10^{-7} and 10^{-8} M concentrations. 1,4-Diaminobutane and 1,5-diaminopentane were also effective, but at much higher concentrations.

Similarly low concentrations of spermidine and spermine were also effective in stabilizing bacteriophage T5 against the rapid loss of titer

TABLE 1

Stabilizing effect of spermine, spermidine, diaminobutane (putrescine) and diaminopentane (cadaverine) on bacteriophage T5 suspended in citrate-containing media

Amine	Molar Concentration	Plaques found (400 at zero time) 0.5 hr.	1 hr.	2 hrs.	4 hrs.	12 hrs.
-	-	15	8	17	14	9
Spermine	5×10^{-7}	385	319	430	433	431
Spermidine	2×10^{-7}	325	263	248	270	169
	5×10^{-7}	382	313	379	398	189
Diaminobutane	5×10^{-7}	17	12	16	14	10
	5×10^{-6}	147	113	71	55	12
	5×10^{-5}	359	314	363	384	378
Diaminopentane	5×10^{-7}	15	10	10	12	15
	5×10^{-6}	105	61	35	27	23
	5×10^{-5}	366	361	410	389	411

The technics used for the preparation and assay of the bacteriophage have been described by Adams (1959). Bacteriophage T5 (A.T.C.C. #11303-B5) was grown on E. coli B in nutrient broth, containing NaCl and $CaCl_2$, to a final titer of 1×10^{10} phage per ml., and was sterilized by filtration. Fifteen μliters of this suspension were diluted immediately before use with 10 ml. of cold 0.001 M potassium phosphate buffer, pH 7.2, containing 500 μgrams of gelatin (Adams, 1948).

The incubation mixtures were prepared at 0°, and contained 10 μmoles of potassium phosphate buffer, pH 7.2, 500 μgrams of gelatin, 20 μmoles of sodium citrate, 5 μliters of diluted phage suspension, and amines (as the hydrochloride salts), as indicated, in a final volume of 10 ml. The mixtures were incubated at 37°, and the phage titer of 0.05 ml. aliquots periodically determined with E. coli B by the soft agar layer technic.

The data are expressed as the number of plaques found with this aliquot, and represent the average of two identical experiments. The zero-time titer, as measured on a comparable dilution with 10 ml. of broth, was 400.

seen in solutions containing sodium ethylenediaminetetraacetate (EDTA). The concentration of EDTA (10^{-5} M) that was effective in causing rapid loss of phage titer was about 100 fold less than that required with sodium citrate.

Discussion

The most striking aspect of the present results is the very low concentration (2×10^{-8} M) of spermine which was effective in stabilizing bacteriophage T5. This concentration was considerably lower than the concentrations of divalent cations (10^{-4} M) that were effective in the experiments of Adams (1949).

Our results should also be contrasted with the experiments of Fraser and Mahler (1958) on a different phage system. These authors used urea-

TABLE 2

The effect of varying concentrations of spermine on the stability of bacteriophage T5 suspended in citrate media

Spermine concentration (moles per liter)	Plaques found (248 at zero time) 0.5 hr.	1 hr.	2 hrs.	4 hrs.	7 hrs.	13 hrs.
0	4[a]	2	5	1	1	1
2×10^{-8}	286	199	194	16	2	1
5×10^{-8}	213	207	185	57	4	2
1×10^{-7}	188	229	237	156	26	5
5×10^{-7}	210	247	265	236	216	190
1×10^{-6}	254	208	295	212	213	180

The conditions were the same as listed in Table 1, except that the initial broth suspension of bacteriophage T5 contained 2×10^9 phage per ml. The final mixtures contained 5×10^3 phage per ml., and 248 phage in the 0.05 ml. aliquot used for the analysis.

a/ The phage titer in the incubation mixtures without spermine decreased to 5 within 5 minutes after incubation at 37°.

shocked phage (π); urea-shocked phage no longer infect E. coli cells, but require protoplasts for infection. Both diaminopentane and spermidine were effective in stabilizing these urea-shocked phage against inactivation by dilution or by heating. Diaminopentane, which was used at a 0.01 M concentration, gave the maximum protective action. In contrast with these results, in the current paper stabilization of bacteriophage T5 was effected by a much lower concentration (10^{-7} M) of spermidine; this concentration is well within the physiological range. Furthermore, in our experiments with bacteriophage T5, diaminopentane was considerably less effective than spermine or spermidine, and did not differ significantly from diaminobutane.

The mechanism of the action of spermine and spermidine in stabilizing bacteriophage is not known. One should note, however, the recent work on the binding of polyamines to nucleic acid (Keister, 1958; Razin and Rozansky, 1959; Felsenfeld and Huang, 1960), and the high concentration of polyamines in certain strains of E. coli bacteriophage (Ames, Dubin, and Rosenthal, 1958; Ames and Dubin, 1960).

REFERENCES

Adams, M. H., J. Gen. Physiol. 31, 417 (1948).

Adams, M. H., J. Gen. Physiol. 32, 579 (1949).

Adams, M. H., Bacteriophages, Interscience Publishers, New York, 1959.

Ames, B. N., Dubin, D. T., and Rosenthal, S. M., Science 127, 814 (1958).

Ames, B. N., and Dubin, D. T., J. Biol. Chem. 235, 769 (1960).

Felsenfeld, G., and Huang, S., Biochim. et Biophys. Acta 37, 425 (1960).

Fraser, D., and Mahler, H. R., J. Am. Chem. Soc. 80, 6456 (1958).

Keister, D. L., Federation Proc. 17, 84 (1958).

Keister, D. L., Ph.D. Thesis, University of Maryland (1958).

Mager, J., Biochim. et Biophys. Acta 36, 529 (1959).

Puck, T. T., J. Bact. 57, 647 (1949).

Razin, S., and Rozansky, R., Arch. Biochem. Biophys. 81, 36 (1959).

Tabor, C. W., Biochem. Biophys. Research Communs. 2, 117 (1960).