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Distinct Effects of Diamines, Polyamines, and Magnesium Ions on the Stability of λ Phage Heads†

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ABSTRACT: The DNA condensed in a tailless λ phage head is readily lost from the structure and appears free in solution. These heads provide a model system for studying interactions in a condensed DNA structure. The diamine, putrescine, stabilizes heads and maintains them in a biologically functional condition but the polyamines, spermidine and spermine, as well as the divalent metal ions, Mg^{2+} and Ca^{2+}, destabilize heads. Putrescine eliminates or moderates the sensitivity of heads to these destabilizing agents. Thus, if the DNA is to remain condensed, the cations that neutralize the DNA phosphate anionic groups also must possess the appropriate steric properties.

Synthesis of the phage head during λ morphogenesis is not dependent upon concomitant synthesis of the tail. Mutants defective in a tail gene produce a full titer of normal heads (Weigle, 1966; Harrison *et al.*, 1973). The stability of these tailless heads differs from that of complete phage. This report describes conditions under which DNA is retained or lost from a head. Of particular interest is the interplay of diamines, polyamines, and metal ions with the phage proteins and DNA. From a description of how they alter stability of the condensed state of λDNA in the phage head, we hope to define parameters that are important for stabilizing the close packing of any DNA.

Materials and Methods

Media and Buffers. K medium is prepared by autoclaving 1 g of NH_4Cl, 6 g of Na_2HPO_4, 3 g of KH_2PO_4, and 1 g of NaCl in 850 ml of glass-distilled water, cooling, and then adding from sterile stock solutions 2 ml of 1 M $MgSO_4$, 10 ml of 2.5×10^{-2} M $CaCl_2$, and 150 ml of 10% Norit A treated Casamino acids. TPBE contains 10^{-2} M Tris-HCl (pH 7.1), 10^{-2} M putrescine-HCl, 10 mg/ml of bovine serum albumin, and 2×10^{-4} M EDTA. TBE has the same composition as TPBE except that putrescine is omitted. Tris–EDTA is 10^{-2} M Tris-HCl with 10^{-3} M NaEDTA, pH 7.1.

Putrescine dihydrochloride, cadaverine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, and all the amino acids except hydroxylysine were purchased from Sigma Chemical Co. and used without further purification. The basic amino acids were obtained as the hydrochlorides and, unless it is otherwise specified, all solutions were tested for their effect on head stability at a pH near neutrality.

Purification of λ Heads. A culture of W3350 *su*⁻ (λ*sus Jam*27) was grown in K medium at 37° to 5×10^8 cells/ml. The cells were chilled, induced with ultraviolet light, and then returned to the shaker bath until the OD at 590 nm indicated lysis was complete. (When preparing heads with ^{3}H-labeled DNA, 1 mCi of [*methyl*-^{3}H]thymidine was added per liter of culutre at 20 min after induction.) After chilling, 1 ml of $CHCl_3$ was added and the debris removed by centrifugation for 45 min at 10,000*g*. In the text, the lysate at this stage of purity is referred to as crude heads. Purified heads were obtained as described by Harrison *et al.* (1973).

Preparation of λ Tails. The source of tails in these experiments was W3350 *su*⁻ (λ*Aam*32 *Bam*1) grown in K medium and induced as described for heads. After the addition of $CHCl_3$ and the removal of cell debris by centrifugation, lysates are stored at 4°. Crude lysates contain 8×10^{10}–2×10^{11} tails/ml and the tails remain active for months. Pure tails are obtained as described elsewhere (Harrison *et al.*, 1973).

In Vitro Head–Tail Joining. Heads are titered by mixing 0.1

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TABLE I: Stabilization of Heads.[a]

Medium	Heads × 10^{-6} 0 hr	48 hr
K medium	470	460
Phosphate buffer	350	0.14
+ 10^{-2} M L-lysine	390	330
+ 5×10^{-3} M L-lysine	390	280
+ 10^{-3} M L-lysine	370	8.3
+ 10^{-2} M hydroxylysine	390	2.4
+ 10^{-2} M L-histidine	450	0.13
+ 10^{-2} M L-arginine	290	0.01

[a] A fresh crude lysate of W3350 *su*$^-$ (λ*Jam*27) was diluted tenfold into K medium or into 10^{-2} M phosphate buffer (pH 7.2) with the indicated amino acid added. The biologically active heads were assayed immediately and after storage for 48 hr at 5°. For assay, heads were diluted tenfold into K medium containing 10^{10} crude tails/ml and incubated 20 hr at 5° before titering for phage.

ml of an appropriate dilution in TPBE with 0.1 ml of crude tails in 0.8 ml of TPBE. In some experiments, K medium was used in place of TPBE. After incubation at 4° for 20 hr the mixture is diluted and plated on the sensitive *Escherichia coli* strain C600. In this study a head is designated as active, or intact, if it combines with a tail to yield a particle that forms a plaque.

Results

The source of heads is a λ lysogen mutant in gene J, one of the phage tail genes. Although these heads are relatively stable in K medium, they can be rapidly inactivated by dilution into phosphate buffer. K is a mineral salts medium buffered by phosphate and supplemented with Casamino acids. When each ingredient of the medium is added to phosphate buffer, only the Casamino acids prevent head inactivation. When each common amino acid is added individually, only L-lysine stabilizes heads. At concentrations above 5×10^{-3} M, the approximate lysine concentration in K medium, it exerts a marked head stabilizing effect. Other basic amino acids are ineffective; see Table I.

TABLE II: Specificity of Head Stabilization.[a]

Compd Added	Surviving Heads × 10^{-6}
No additions	1.0
L-Lysine	83
D-Lysine	1.9
L-Ornithine	0.7
D-Ornithine	1.5
Putrescine	220

[a] Purified heads in TPBE sucrose were diluted 1000× into 10^{-2} M Tris-HCl (pH 7.1), 10^{-2} M NaCl, and 10^{-4} M $MgSO_4$ with the indicated diamino compounds added to 10^{-2} M (carryover of putrescine with heads is 10^{-5} M). After incubation for 30 min at 37°, the heads were diluted 100× into TPBE containing a tenfold diluted crude tail lysate and incubated 20 hr at 5° before titering for completed phage particles. No loss of active heads occurred in 30 min when putrescine was added and its titer is also the number of heads at zero time.

TABLE III: Effect of Polyamines on Head Stability.[a]

Additions	Concn (M)	Active Heads (pfu × 10^{-6}) 30 sec	2 hr	4 hr
None		188	51	31
1,3-Diaminopropane	10^{-2}	221	156	150
	10^{-3}	237	122	146
	10^{-4}	199	77	68
1,4-Diaminobutane	10^{-2}	216	222	199
(putrescine)	10^{-3}	193	166	156
	10^{-4}	198	165	102
1,5-Diaminopentane	10^{-2}	212	136	70
(cadaverine)	10^{-3}	234	93	85
	10^{-4}	198	66	49
Spermine	10^{-4}	32	2	2
	10^{-5}	143	7	5
	10^{-6}	241	66	38
Spermidine	10^{-4}	178	8	6
	10^{-5}	199	61	40
	10^{-6}	224	57	41

[a] Purified λ*Jam*27 heads ($2–3 \times 10^{11}$/ml) in TPBE sucrose were diluted 100× in buffer (10^{-2} M Tris-HCl (pH 7.1), 10^{-1} M NaCl, and 2×10^{-4} M EDTA) containing the indicated additions. Samples were diluted and assayed as in Table II.

In addition to amino acids, a number of diamines were examined for their ability to alter head stability. Among those tested, putresine, $NH_3^+(CH_2)_4NH_3^+$, is the most effective stabilizing agent. It is superior to L-lysine. Ornithine, the amino acid analog of putresine, and D-lysine are unable to stabilize effectively; see Table II.

We have examined the effect of varying the carbon chain length between amino groups of diamines, and more extensive data and a discussion of it will be presented in a subsequent paper (Harrison, D. P., and Bode, V. C., manuscript in preparation). The four-carbon diamine, putrescine, and the three-carbon diamine, 1,3-diaminopropane, both stabilize optimally; nevertheless, the polyamines which have similar numbers of carbons between amino groups do not. Spermidine, $NH_3^+(CH_2)_3NH_2^+(CH_2)_4NH_3^+$, a natural polyamine of *E. coli*, and spermine, $NH_3^+(CH_2)_3NH_2^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$, a polyamine found in many other cells, destabilize head; see Table III. Under these conditions the effect of these polyamines is on the stability of heads not on the joining reaction itself. This is indicated by the zero time samples, most of which have high head titers but the same carryover of polyamine as the later samples. At high concentrations of spermine even the initial sample is of low titer. Diluting the heads an additional tenfold before joining with tails does not increase this titer, suggesting that the inactivation is rapid and indeed occurs prior to dilution for assay.

Bovine serum contains a diamine oxidase (Tabor *et al.*, 1954) which oxidizes spermine, but not putrescine, to an aldehyde that inactivates many phages including λ (Bachrach and Leibonici 1965, 1966; Fukami *et al.*, 1967). Since TPBE contains a large amount of bovine serum albumin, we performed a control experiment to eliminate the trivial explanation for

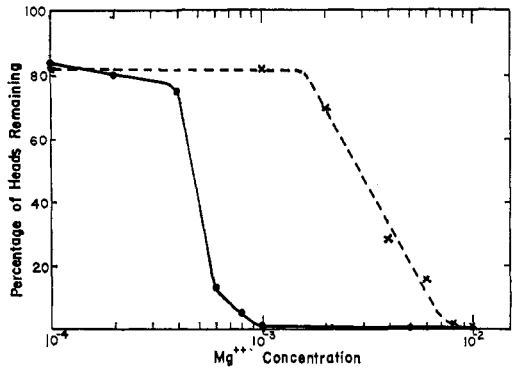

FIGURE 1: The effect of putrescine concentration on Mg^{2+} ion promoted head inactivation. Purified heads were diluted at least 100-fold from a TPBE solution containing about 25% sucrose into buffers containing 10^{-2} M Tris (pH 8.0), 10 mg/ml of bovine serum albumin, the indicated $MgSO_4$ concentration, and enough putrescine to yield a final concentration of 10^{-4} M (—●—) or of 10^{-3} M (--×--). The solutions were incubated at 0° and the biologically active heads assayed after 30 sec, 30 min, and 1 hr as described in Table III. The per cent of heads which were active after incubation for 30 min is plotted as a function of the Mg^{2+} concentration. Additional inactivation occurs with time.

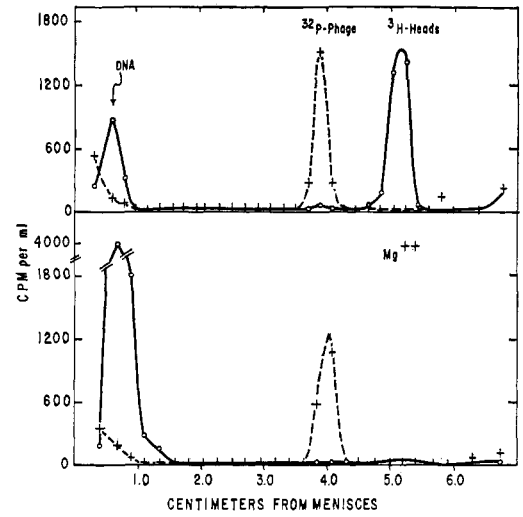

FIGURE 2: The loss of DNA from phage heads on exposure to Mg^{2+}. ^{3}H-Labeled phage heads were purified as described under Methods except 10^{-2} M Tris-HCl-10^{-2} M lysine-10^{-1} M NaCl (pH 8.0) was used throughout in place of TPBE. The heads were diluted in the lysine-containing buffer to 10^{10}/ml (6000 cpm/ml) and ^{32}P-labeled marker λ phage was added (3000 cpm/ml). A portion of the solutions was made 10^{-2} M in $MgSO_4$. Before and after the addition of Mg^{2+} samples (1 ml) were layered on sucrose gradients (15–35% sucrose in the Tris-lysine-NaCl buffer) and centrifuged for 90 min at 25,000 rpm in the SW25 spinco rotor. Fractions of about 1 ml were collected, the labeled DNA and phage were precipitated in 5% trichloroacetic acid, and the precipitated material was collected on a glass fiber filter and then counted. All fractions were assayed but baseline data points are not indicated.

polyamine inactivation, namely, that the albumin was contaminated with amine oxidase activity. Heads purified and incubated with polyamines in the absence of albumin were readily inactivated by spermine and spermidine.

The presence of divalent metal ions in a solution can greatly enhance the rate of head inactivation. The concentration dependence of Mg^{2+}-promoted head inactivation is a function of the putrescine concentration. Thus, in 10^{-3} M putrescine, heads are relatively stable to 10^{-3} M Mg^{2+} but in 10^{-4} M putrescine they are not; see Figure 1. For head stability, the ratio of divalent metal ion to diamine concentration is more crucial than is the absolute concentration of either. Completed phage, in contrast to heads, are more stable over long periods of time if magnesium is present.

The decrease in the number of biologically functional heads that is observed after Mg^{2+} (or Ca^{2+}) addition is associated with the leakage or expulsion of DNA from the tailless head; see Figure 2. Heads containing [^{3}H]thymidine-labeled DNA were sedimented with a marker phage whose DNA contained ^{32}P. Heads sediment faster than ^{32}P-labeled phage particles since the long tail slows the latter. If the heads are exposed to Mg^{2+}, the labeled DNA is lost and, after centrifugation, is found near the top of the gradient sedimenting at the rate of free λDNA molecules rather than at the rapid rate characteristic of heads. As judged by experiments similar to the one described in Figure 2 for Mg^{2+} ions, spermine and spermidine inactivation of heads also is associated with a loss of DNA from the heads.

As with Mg^{2+}, inactivation by polyamines is dependent upon the putrescine concentration. For example, with 10^{-4} M putrescine in the Tris–albumin–EDTA buffer, 50% inactivation occurs in 1 hr if either 5×10^{-5} M spermine or 5×10^{-4} M spermidine is present. When the putrescine concentration is increased tenfold to 10^{-3} M, the spermine concentration yielding 50% inactivation in 1 hr is increased by 100-fold to 5×10^{-3} M. Thus, moderate levels of putrescine protect well against inactivation by reasonable concentrations of spermine and spermidine, but in the absence of an appropriate diamine, heads are inactivated by very low concentrations of polyamines.

Discussion

λDNA is condensed in the phage head with different parts of the polyanionic phosphodiester backbone positioned very close to each other. Since micrococcal nuclease treatment of tailless heads removes the four terminal bases from the right-hand single-stranded end of every packaged DNA molecule (Bode and Gillin, 1971; Gillin and Bode, 1971; Padmanabhan *et al.*, 1972), the packing probably is uniform in every head with the same end precisely positioned at the tail attachment site. Various diamines, polyamines, proteins, and ions may facilitate and stabilize the close and specific packing of DNA. This involves, in part, neutralizing the charges on the DNA phosphates.

In the following discussion, we assume that the cation specificity for head stability resides predominantly in interactions with the phage DNA. At present, we know of no way to exclude the alternate possibility that they act directly on one or more proteins at the tail attachment site and that this triggers the loss of DNA. Several points can be made: (1) none of the compounds, even at 10^{-2} M, inactivate phage; (2) the compounds are known to bind tightly to DNA; (3) their effects on heads occur at very low concentrations in the absence of putrescine protection; and (4) phage whose heads are not permeable to putrescine or spermidine contain them as counterions for the DNA phosphates (Ames and Dubin, 1960).

In most studies of the binding of diamines, polyamines, and metal ions to native DNA or synthetic polynucleotides, the primary concern has been the tightness of binding or the stabilization of the double helix against melting or shear. (See the recent excellent review of polyamines by Tabor and Tabor (1972) or papers by Herbst and Bachrach, 1970.) In some studies polyamines stabilized more complex structures where tertiary structure is important, *e.g.*, T-RNA (Cohen *et al.*,

1969), ribosomes (Cohen and Lichtenstein, 1960), osmotically sensitive phage (Tabor, 1960), or urea-shocked phage (Fraser and Mahler, 1958). In this study, it is the tertiary or folded DNA structure that must be maintained and in contrast to the general stabilization theme in the above studies our data show that Mg^{2+}, spermine, and spermidine destroy head structure by the loss of DNA.

One can formulate models where stabilizing the DNA helix might destabilize heads. Optical and chemical reactivity studies have suggested that packing DNA in some phage heads involves both the unstacking of part of the phage DNA and the noncovalent interaction of the unstacked bases with proteins (Tikchonenko *et al.*, 1973). X-Ray studies suggest that spermine can bind across the minor groove bonding to two phosphates on each strand and stabilizing the duplex. It may also bind in other ways (Liquori *et al.*, 1967b; Suwalsky *et al.*, 1969). Liquori and coworkers (1967a) report that the binding of spermine competes with the ability of DNA to solubilize 3,4-benzpyrene. Presumably the solubilization occurs by the intercalation of benzpyrene within disordered regions of the helix and spermine interferes because it re-orders these regions reducing intercalation. In a similar way polyamines might reorder the helix in the phage head and prevent interactions between unstacked bases and phage proteins, thereby destabilizing condensed head DNA. No specialized internal proteins are known for λ but interactions between DNA and head structural proteins, or between DNA and a specific protein in the head–tail connector, probably occur.

Normally *E. coli* contains the polyamines putrescine and spermidine in a molar ratio of 3:1. The data of Tabor and Tabor (1969) place the potential intracellular concentration of putrescine at 10–20 mM. Even if a significant per cent of this is bound, the remaining free diamine would provide significant *in vivo* stabilization for λ heads.

The instability of phage heads in the presence of spermine may be a property of certain other phages. Ames and Dubin (1960) infected *Salmonella typhimurium* growing in medium containing 1 mM spermine with P-22 phage. They were unable to obtain phage from these bacteria so long as spermine was present. Although a number of alternative explanations are equally possible, this is the expected result if P-22 heads are as sensitive to inactivation by spermine as are λ heads.

If we are correct in assuming that the molecules studied stabilize or destabilize heads as a consequence of their binding to DNA, their effects might be rather general for other structurally labile but closely packed DNA structures.

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