The Thermal Lability of Tryptophan-dependent Bacteriophages

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SUMMARY

Bacteriophages which are tryptophan-dependent for adsorption are also sometimes heat-labile in the presence of added tryptophan. The inactivation is due to the discharge of the phage deoxyribonucleic acid (DNA). This phenomenon is most marked at two different monovalent ion concentrations and is thought to be due to production of lesions at two different sites on the phage tail.

INTRODUCTION

Cheng (1956) reported that the 'tryptophan-dependent' phage T4,38 was inactivated by heating to 53.5° for 1 hr., but only in the presence of tryptophan. Fildes & Kay (1959), using a similar phage, obtained the same result on heating to 45°. Electron micrographs showed disintegration of the tail tip. These phages belong to a well-defined group in which the adsorption factor requirement is not constant since tryptophan-independent mutants (tryp⁻) are easily prepared and indeed tryptophan-requiring mutants (tryp⁺) of at least one, T2, are unknown. The group is defined by containing hydroxymethylcytosine (HMC) in its deoxyribonucleic acid (DNA) and is morphologically homogeneous (Bradley & Kay, 1960). The present paper explores the heat lability of these phages further.

MATERIALS

The HMC phages known to us are the three T-even phages and others isolated from sewage (Kay & Fildes, 1961) together with several mutants which have appeared during the present work. The direct effect of tryptophan can, of course, only be demonstrated with tryp⁺ specimens which require its addition. It may be stated at once that the heat-labile phage referred to by Fildes & Kay (1959) has proved to be the only frankly labile phage in our collection. This character is however reproduced in certain mutants.

This heat-labile phage is referred to in this paper as 'K18/3' and its derivation is as follows:

A phage was isolated by us in 1954. This was described by Fildes & Kay (1957). During 1955 and 1956 four serial propagations were made through various strains of *Salmonella typhi* under different conditions. These lysates were and are heat stable. During 1957 and 1958 a further passage was made under similar conditions and four further serial passages through a sensitive *Escherichia coli* in a glucose-ammonia

medium. The final passage K18/3/59 showed abnormalities which led to recognition of the fact that it was heat-labile. No selective pressure was applied to favour heat-labile progeny and thus it is improbable that K18/3 is a rare mutant of the original phage. Rather it would seem that it is a variant produced suddenly or gradually by changing conditions of propagation. We have been unable to reproduce this change in controlled experiments.

METHODS

The general procedure is indicated in the protocols. It follows that of Fildes & Kay (1957), but, to remove a disturbing factor, phosphate buffer was replaced by 0.011 M-tris buffer (2-amino-2-hydroxymethylpropane-1:3-diol) at pH 7.6. All solutions were made up in this buffer, which contained, as before, human albumin. DL-tryptophan was used throughout.

RESULTS

The effect of monovalent cations in the heat/tryptophan inactivation

The labile phage under discussion is referred to as 'K18/3'. This phage when suspended in 0.011 M-tris buffer and tryptophan could be heated to 45° for 1 hr. without inactivation. It was found that inactivation required also the presence of monovalent cations. An experiment was arranged as follows. Decreasing volumes of 0.25 M-NaCl in 0.011 M-tris buffer were balanced with increasing volumes of 0.011 M-tris buffer to give a series of NaCl concentrations ranging finally from 0.113 M to 0.0124 M. These were made in 1 ounce screw capped bottles and DL-tryptophan was added to 10^{-4} M, leaving a control series without tryptophan and 1 control bottle containing no NaCl. All received phage K18/3 to make 2.5×10^8 phage particles/ml. final. The bottles were immersed in water at 40°. After 30 and 60 min. the phage content was assayed by the drop method. The measure of the input phages was taken as the average of the bottles at time 0. This figure was found to be 333 phages in 4 drops (0.1 ml.). The counts of the 8 bottles containing no tryptophan ranged from 342 to 296; that of the bottle containing tryptophan but no NaCl, 321; while the results of the tryptophan-containing bottles are shown (Fig. 1) as percentage inactivated at various NaCl molarities. This experiment showed that phage K18/3 at 40° in the presence of tryptophan and NaCl was heat labile, but stable for 60 min. in the absence of either tryptophan or NaCl.

Figure 1 shows that there was a peak effect at 0.02 M-NaCl and a depression at 0.05 M, followed by a rise up to 0.12 M-NaCl. The shape of this curve of NaCl titrations is constant. Figure 1 however does not show clearly the effect of concentrations of NaCl less than that at the peak. This is indicated in Fig. 2. The percentage inactivation in 60 min. at 40° has a linear relationship to the concentration of NaCl.

In these experiments the reacting substances were confined to phage, tryptophan and NaCl at a constant pH and temperature. There was no evidence that variation in the concentration of phage or of DL-tryptophan above 2×10^{-5} M had any bearing on the result. Thus the variations in percentage inactivation must be due to changes in the concentration of monovalent cations.

The action of other monovalent cations in the presence of tryptophan was also tested. Tris buffer at 0.011 M had, as already stated, no measurable action in 60 min.

at 40° and at 0.02M the effect was very slight. However, potassium, rubidium and lithium were all active. Figure 3 shows that the action of lithium resembled that of sodium (Fig. 1).

The effect of divalent cations on the heat/tryptophan inactivation of phage K18/3

While the monovalent cations at certain precise concentrations are essential for a high rate of inactivation of this phage, the divalent cations are inhibitory by reason, apparently, of some relationship between the monovalent/divalent ion concentrations. The data already shown in Fig. 2 were used to show the percentage inactivation in 1 hr. at 40° due to different concentrations of NaCl. In two further experiments the percentage inactivation was determined in the presence of various concentrations of

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Effect of NaCl on inactivation of phage K18/3 for 60 min. at 40°.

Fig. 2. Inactivation of phage Y in 60 min. at 40°. Fifteen points from four experiments. Phage Y was derived from phage K18/3 and is equally heat sensitive.

Fig. 3. Effect of lithium on inactivation of phage K18/3 for 60 min. at 40°.

magnesium ions and a constant concentration of NaCl approximating to the peak, 0.022M (Fig. 2). These results are shown in Fig. 4. It was now argued that if a certain concentration of MgSO_4 + an optimum concentration of NaCl produced the same degree of inactivation as a certain concentration of NaCl alone, it could be said that, for instance, 0.0222M-NaCl in the presence of 0.0001M- MgSO_4 left a surplus of NaCl capable of producing 61% inactivation. From Fig. 2, this degree of inactivation was given by 0.0165M-NaCl and so it could be calculated that 0.0001M- MgSO_4 was 'equivalent' to $0.0222 - 0.0165 = 0.0057$ M-NaCl. Molarities of MgSO_4 plotted against their 'equivalents' of NaCl obtained in this way are shown in Fig. 5. It will be seen that the ratios Mg/Na vary from 1:74 to 1:34, but taking into account the technical difficulties it seems likely that the 'equivalence' ratios of the two cations are really constant.

The temperature range of the tryptophan/heat inactivation of phage K18/3

These experiments were carried out as follows. Two screw cap bottles were set up, both containing NaCl 0.025M (final) and DL-tryptophan 10^{-4} M (final). Volumes being equalized with 0.011M-tris buffer, phage K18/3 was added to 2.5×10^3 particles/ml.

final. The bottles were then immersed in water at 24°. Samples were assayed for phage at 0, 10, 20, 30, 40 and 60 min. The average of the counts of all the readings without tryptophan was taken to be the input; this was 290 plaques/0.1 ml. The individual counts of all plates were then plotted as log % input surviving at times stated (Fig. 6). It is seen that in this case 32 % of the input phage had been destroyed in 60 min. with tryptophan, but none without. Table 1 summarizes the results at various temperatures with tryptophan; no inactivation occurred without tryptophan. At 50° inactivation with tryptophan present was 99 %, but 80 % without tryptophan. Between 45 and 50° therefore a specific effect of tryptophan on heat inactivation was not demonstrable.

Fig. 4

Fig. 5

Fig. 6

Fig. 4. Effect of magnesium on inactivation of phage Y in presence of 0.022M-NaCl for 60 min. at 40°. Combined results of two experiments.

Fig. 5. 'Equivalence' between Mg and Na affecting the inactivation of phage Y.

Fig. 6. Rate of inactivation of phage K18/3 at 24° without tryptophan (O), with tryptophan (●).

Table 1. *Effect of different temperatures on the degree of inactivation in 1 hr.*

Temperature	Inactivation (%)
19	21
24	32
30	40
36	65
37	64
42	86
45	87

Discharge of DNA in tryptophan/heat inactivation of phage K18/3

As shown by Cheng (1956) the phage DNA is discharged during inactivation. Its release can be observed conveniently by the concomitant increase in viscosity of the solution. Four mixtures were made in screw-capped bottles all containing tryptophan. Bottle A contained finally 0.022M-NaCl, Bottle B 0.054M, Bottle C 0.155M and D no NaCl. It will be noticed that the three concentrations corresponded with the first peak, depression and second rise of Fig. 1. Phage K18/3 was added to give a count of 1.1×10^{11} particles/ml. Counts were made of the input phage at time 0 and of the survivors after 60 min. at 40°. Specimens were placed in Ostwald-type viscometer tubes at 40° at time 0 and flow-times were repeatedly measured.

The counts after the experiment showed that in Bottle A 95 % of the input had been inactivated; in Bottle B 40 %; in Bottle C 99 %; in Bottle D there was no inactivation. Figure 7 shows the rate of discharge of DNA and its dependence on the same conditions as inactivation, namely the presence of suitable concentrations of

Fig. 7. Release of DNA from phage K 18/3 tryptophan mixtures at different concentrations of NaCl. Increase in flow-time in viscometer tube at 40°. No NaCl, (○); 0.022M-NaCl, (△); 0.054M-NaCl, (△); 0.115M-NaCl, (●).

Fig. 8. Effect of varying NaCl concentrations on the inactivation of phage Y (○) and phage ZH (●) in 60 min. at 40°.

NaCl. The inactivation of phage by heat/tryptophan is known to be associated with anatomical changes in the phage tail which no doubt are the immediate causes of the discharge of DNA. It seems however probable that the anatomical changes are not the same at all NaCl concentrations and that the depression in the middle of Fig. 1 is due to a change in the quality of the lesion.

The behaviour of certain mutants of phage K18/3

Phage K18/3 is a direct descendant of the original 'Phage 3' isolated by us in 1954. The original phage and a recent repropagation under current conditions were heat stable. Four serial propagations of this phage for stock purposes remained heat stable but after five further propagations it was noticed that the heat-labile strain (K18/3) had appeared. It was found that isolated plaques behaved differently with isolated colonies of the coli cultures used. In this way two different phages were separated on host-range differences. One of these, 'Y', was sensitive to 37°; the other, 'ZH', was much more stable.

Fig. 9. Effect on inactivation of phage ZH of prolonged time at 40°. 1 hr. (O), 2½ hr. (●), 5 hr. (Δ).

Phage K18/3 was heated in the presence of tryptophan and 0.025M-NaCl for 12 hr. at 45°. At the beginning there were 1.3×10^{10} phages/ml.; at the end 3.1×10^7 . This remnant propagated in the usual way produced a lysate from which plaque selection was made and phage F.H45.T produced. This phage proved to be stable at 37° for 40 min.

The two phages thus separated from K18/3 were more heat stable than the rest of the population. When phage ZH was tested for stability at various NaCl molarities it was found (Fig. 8) to show a small but sharp rise in lability at the same peak (0.027M) as its fellow Y; but the secondary rise in the range 0.11M did not appear or at least was insignificant. When also the action of heat on phage ZH was more prolonged, i.e. to 5 hr. (Fig. 9), no less than 84% was inactivated at 0.027M-NaCl, but still the secondary rise was much less than in the heat-labile variety (cf. Figs. 1, 8, 9). It thus appears that the more heat-stable phages may differ from the more labile in two ways: firstly, in being slower to respond to the heat inactivation at 0.027M-NaCl and, secondly, in being less susceptible to the inactivation at 0.11M-NaCl.

DISCUSSION

The property of marked heat-lability in the presence of tryptophan and monovalent ions does not seem to be widespread among the HMC phages. In our collection only one out of twelve phages isolated from sewage was sensitive (excluding its mutants) and Cheng (1956) mentioned only one phage of this type. Our phage K18/3 only shows its lability when the ionic environment is precisely adjusted and when tryptophan is present. Otherwise it is as stable as its parent strain. In any case the stability to heat is a relative matter. Comparison between our stable and unstable variants isolated from K18/3 shows that the difference is one of rate of inactivation rather than of complete resistance or complete sensitivity. A more subtle difference between the two phages is revealed when their inactivation is studied with respect to the monovalent ion concentration. It is then seen that even when the degree of inactivation at the low NaCl concentration is increased by prolonging the time of the experiment the inactivation at the high NaCl concentration does not increase to that of the labile phage variant. We therefore conclude that though the actions of NaCl at the high and low concentrations are similar, namely to produce a lesion in the presence of tryptophan which results in discharge of DNA from the phage, nevertheless the actions at the two NaCl concentrations are exerted at different sites in the phage tail.

Our findings on the effect of monovalent and divalent salts on the action of heat and tryptophan on HMC phages are, broadly, similar to those of salts and heat on phages which are indifferent to tryptophan (cf. Gard & Maaløe, 1959). The lesions produced by heat in a certain ionic environment may well be the same with many phages. But in the HMC phages tryptophan is a necessary activator of the action.

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