SIZE OF DNA MOLECULES IN BACTERIOPHAGE T_7 AFTER ^{32}P DECAY

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Since the first studies by Hershey et al. (1951) the inactivation efficiency of ^{32}P transmutation in bacteriophages has been measured many times. The gathering of values very close to 0.1 for phages having double stranded DNA led to the hypothesis (Stent and Fuerst, 1955) that DSB[1] are responsible for inactivation of phages and that ^{32}P transmutation generates DSB with a probability of 0.1 . This theory was supported by the 100% efficiency of ^{32}P suicide in phages possesing single stranded DNA (Stent and Fuerst, 1960). However, there was no direct proof of DSB after ^{32}P transmutation. Recently, Ogawa and Tomizawa (1967) showed that ^{32}P decay converts superhelices of lambda DNA into linear molecules with a probability close to 0.1 .

Our present results show that the probability of DSB formation by ^{32}P transmutation in DNA of phage T_7 is much lower than 0.1. In addition we are able to prove that no natural SSB[1] exist in DNA of T_7 .

Methods: Bacteria Escherichia coli B 228 were grown on glycerol-lactate medium (Hershey et al., 1951) containing 2 mg P / l. An overnight culture was diluted 20-times and was shaken 90 minutes. Carrier-free ^{32}P was added at concentrations of 100 μc/ml and 0.235 μc/ml and the cultures were infected by bacteriophage T_7 with a multiplicity of 0.2 . After another 90 minutes of shaking the lysis was completed by chloroform and phage particles were isolated by differential centrifugation and sedimentation in CsCl gradients. Specific activity of ^{32}P in the phage DNA was checked by measuring viable phages (the survival after purification was about 95%) and absolute dosimetry.

[1] DSB = double strand breakes, SSB = single strand breakes

Carrier phage was added and part of the suspension was stored in a frozen state over dry ice and another part was used for DNA isolation by the phenol method (Thomas and Abelson, 1965).

Isolated DNA was analyzed by zone centrifugation using neutral (1M NaCl, 0.01M tris, 0.001M EDTA, pH 7.5) or alkaline (0.1M NaOH, 0.9M NaCl, 0.001M EDTA) sucrose (5 - 20%) gradients. In some experiments phage particles were layered directly into the 0.1 ml of 0.5M NaOH floating on the top of the alkaline gradient (McGrath and Williams, 1966). The resulting sedimentation curve does not differ from the profile obtained with isolated DNA. The DNA samples stored after isolation in buffer (pH 7.2) saturated with phenol do not differ in sedimentation pattern from samples freshly isolated from phage stored over dry ice.

Results: Sedimentation curves of phage DNA are given in Figs. 1 and 2. It can be seen from Fig. 1 (curve a) that the DNA from the experiment with low specific activity gives a sharp single peak. This result can be interpreted in the following way:

a) there are no natural SSB in the DNA of T_7 phage, or

b) SSB are present in less than 2% of phages (this uncetainity is due to the limits of our method), or

c) SSB are present in all molecules of phage DNA and are situated near the end of the strand. If so the big piece (e.g., 98% of the whole molecule) cannot be resolved from the main peak and the small piece (e.g., 2%) has the activity which is below our experimental error. Pieces larger than 2% of whole molecule should be detected.

Decrease of molecular weight of single stranded DNA occured in the experiment with highly labelled DNA even in the sample analyzed in the shortest time possible (12 hours from infection of the labelled culture) (Fig. 1, curve b). The shape of the curve allows us to estimate the efficiency of transmutation in generating SSB:

At time t = 12 hours there are in average 0.5 atoms decayed per one strand of T_7 DNA labelled with specific activity 176 mc/mg P (molecular weight of T_7 DNA is taken 2.4×10^7 (Abelson and Thomas, 1966). Using

Poisson distribution we may estimate the fraction of strands where no transmutation occurred

$$N_0 = e^{-0.5} \doteq 0.6$$

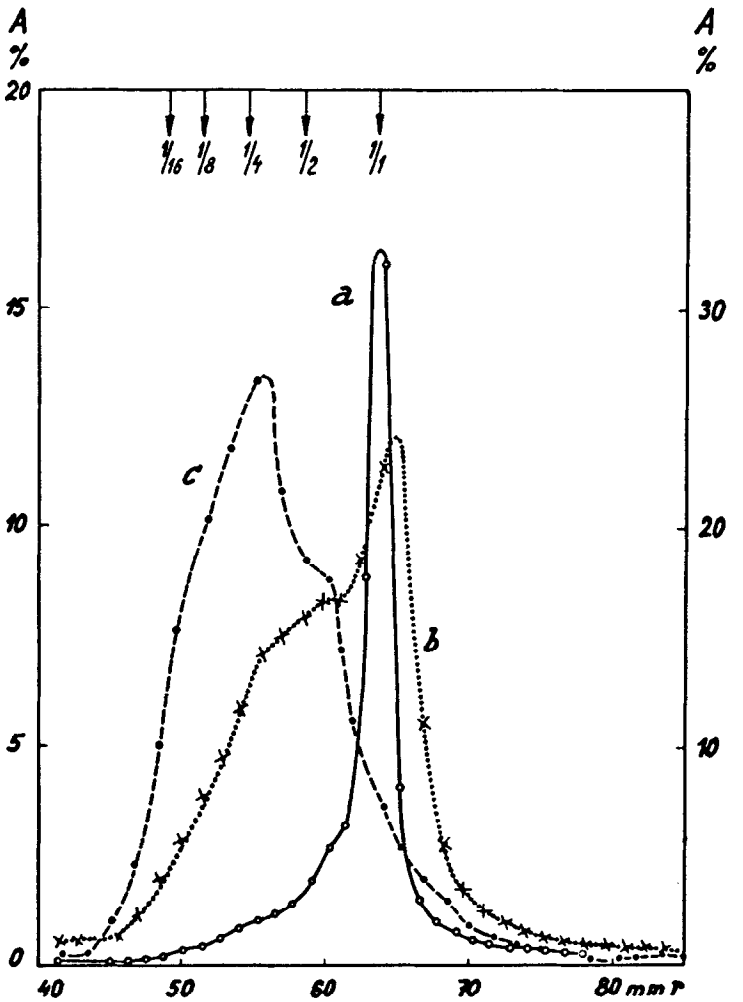

Fig. 1. Sedimentation patterns of T_7 DNA in alkaline sucrose gradients:
a - DNA labelled 0.42 mc/mgP at time t = 19 hours
b - DNA labelled 176 mc/mgP at time t = 12 hours
c - DNA labelled 176 mc/mgP at time t = 24 days
Arrows on the top give possitions of fragments (1/2, 1/4, .., etc) of whole molecule calculated according to the relation $D_1 : D_2 = (M_1 : M_2)^{0.35}$ (Burgi and Hershey, 1963).

If the curve b in Fig. 1 is resolved to isolate the main peak the aerea of this peak corresponds to 55.7% of total activity. Thus, we may conclude that all ^{32}P transmutations cause SSB.

After 69% of ^{32}P had decayed the average molecular weight of the separated strands is reduced by a factor greater than 4 (Fig. 1, curve c).

On the contrary, no decrease in molecular weight was detected in neutral sucrose gradients. The fraction of activity found on the low molecular weight part of the curve is 2.2% of total activity. If all this activity is

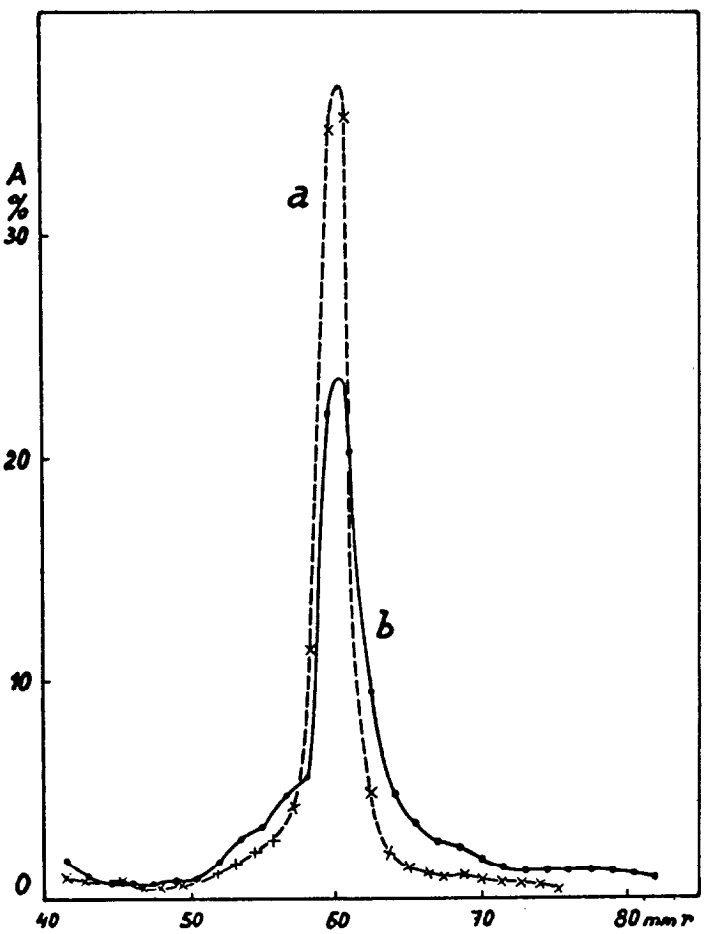

Fig. 2. Sedimentation patterns of T_7 DNA in neutral sucrose gradients:
a - DNA labelled 176 mc/mgP at time t = 17 hours
b - DNA labelled 176 mc/mgP at time t = 24 days

attributed to broken molecules, there will be on the average 0.022 DSB per one molecule. However, this is the highest estimate since a great deal of this activity is due to imperfections of our method (e.g., shearing during isolation, broadening of the band due to greater volume of the sample, etc.). From this estimate and the known specific activity we may conclude that the efficiency of ^{32}P transmutation in generation of DSB is lower than 0.0076 DSB per transmutation.

From our results we can conclude:

1) We are not able to find the heterogenity of denaturated T_7 DNA which was described by Freifelder (1966, 1967) and Davidson et al. (1964). Our finding is in good agreement with Abelson and Thomas (1966) and Thomas (1967).
2) The efficiency of generation of DSB by ^{32}P transmutation is more than one order lower than the killing efficiency (Stent and Fuerst, 1960).

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