Micrococcal Nuclease Treatment of Bacteriophage Heads Alters the Right-Hand Cohesive End of Lambda Deoxyribonucleic Acid

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Isolated bacteriophage lambda heads were exposed to micrococcal nuclease prior to addition of the phage tail. The deoxyribonucleic acid (DNA) was extracted from the heads and sheared to half-molecules whose cohesive ends were annealed to normal lambda DNA half-molecules. Melting curves of each of the cohered halves indicated that only the right-hand termini are altered by nuclease treatment.

Lambda phage heads have an opening, presumably at the site of head-tail joining, where the encapsulated deoxyribonucleic acid (DNA) can be attacked by micrococcal nuclease (MN). Tails join normally to nuclease-treated heads but produce defective phage whose adsorption and DNA injection are normal but whose specific infectivity is only 5 to 15% that of control (1). DNA was extracted from MN-treated and control λ heads (MN-DNA or control DNA), and the nature of the nuclease damage was determined (2). Those results are summarized in Table 1. Sedimentation of MN-DNA in alkaline sucrose gradients revealed no hydrolysis of internal phosphodiester bonds, indicating the site of damage was near the ends of the DNA molecule. Whole or half-molecules of MN-DNA were as efficient as control DNA in transformation with helper-infected cells. Since a relatively intact cohesive end is essential in this system, it was argued that the nuclease damage must be very limited. In agreement with this idea, MN-DNA readily formed circles *in vitro* by hydrogen bonding at its complementary single-stranded ends. The circle melting temperature, that temperature (T_m) at which 50% of the DNA is linear duplex and 50% is circular duplex, of the MN-DNA circles was 36°C, whereas that of control DNA circles was 61°C. This suggested that fewer base pairs were available to maintain the circular conformation and that several terminal bases of the single-stranded 5' ends had been removed from the packaged DNA during the MN attack on heads.

The following experiment was designed to determine whether bases are removed from both ends of the molecule, from one unique end, or from one end chosen at random. A λ right half-molecule, carrying the immunity region, *i*, can anneal specifically with a left half-molecule, carrying genes A and B, by hydrogen bonding between the single-stranded ends (3). After annealing, the transformation activity of the *i*⁺ or *sus* A⁺B⁺ genes is reduced to approximately 30% that of the original halves or of halves which are separated again by heating. MN or control DNA halves, genotype *i*⁺ and *sus* A⁺B⁺, were joined with a 20-fold excess of genetically heterologous (*i*⁴³⁴ and *sus* AB) λ DNA half-molecules. At this ratio of DNA concentrations, 95% of the MN (or control) DNA right half-molecules should be joined with an unaltered left half. Analogously, MN-DNA left half-molecules (*sus* A⁺B⁺) anneal predominantly with unaltered (*i*⁴³⁴) right half-molecules. (The excess *i*⁴³⁴ and *sus* AB right and left halves anneal with each other but lack genetic markers that will be subsequently detected by the indicator strains.) Thus damage to the MN-DNA ends should be reflected in deviant melting behavior (5, 6) that can be monitored by transformation. If MN damages both ends of each λ DNA molecule, then both halves should have a T_m lower than the control but higher than 36°C, since each half is now annealed with a normal half. If one unique end is damaged, then that half-molecule should have a low T_m , near 36°C, whereas the other half should exhibit normal melting, near 61°C. If one randomly selected end is damaged, then the melting curves for both half-molecules should be biphasic, reflecting damage to 50% of the right ends and to 50% of the left ends.

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TABLE 1. Properties of DNA extracted from micrococcal nuclease-treated heads

1. Single-strand molecular weight.	Normal ^a
2. Transforming activity (whole or half-molecules).	Normal ^a
3. Rate of formation of hydrogen-bonded circles at 10 C.	7X faster than control ^a
4. T_m of hydrogen-bonded circles.	25 C lower than control ^a

^a The control is DNA extracted from λ heads subjected to identical treatment except that no micrococcal nuclease was present during the incubation. For details of these experiments see reference 2.

Melting curves of each half of the MN, or control, DNA were obtained by assaying the transformation activity of the genes i^h (right half) and $sus A^+B^+$ (left half) in annealed DNA samples which had been heated to a given temperature and cooled quickly. The T_m calculated for both the right and left halves of control DNA is approximately 58 C (Fig. 1A). The melting curve (Fig. 1B) of the left half of the MN-DNA molecule, measured as $sus A^+B^+$ activity, exhibits a T_m of 58 C, identical to that of undamaged control DNA. Limited damage to the left half-molecule might not be detected by this method as it is possible that removal of only one base would not change the melting curve. In contrast, the i^h activity of MN-DNA associated with the right half increases between 30 C and 42 C and then decreases (Fig. 1B). If the increase in activity represents melting, then the T_m is 36.5 C and the data indicate that the altered melting and annealing behavior of MN-DNA molecules is due to changes in the right end of the molecules only.

Two factors may have contributed to the apparent decrease in transformation when annealed DNA was heated above 50 C. The decrease in MN-DNA right half activity occurs only after heating to the temperature range where the normal annealed halves melt. There is a 20-fold excess of self-annealed heterologous DNA whose ends melt at these temperatures so that it becomes available to the transformation system. Although the heterologous DNA is not assayed because of its genotype, it can compete in the assay with the previously melted MN-DNA half if the DNA concentration is high. Very high DNA concentrations were used in these experiments because the efficiency of the $sus A^+B^+$ assay for the left half was very low. Therefore, assays were performed above the linear dose-response range of total λ

FIG. 1. Melting curves for micrococcal nuclease (MN) and control DNA half-molecules (i^h $sus A^+B^+$) hydrogen bonded to λi^{434} $sus AB$ half-molecules. DNA was extracted from λ heads which had been subjected to MN or control (lacking enzyme) treatment (1). DNA half-molecules were produced by shearing the DNA at 1,200 rev/min for 30 min at 0 C. The i^h gene is on the right DNA half-molecule whereas genes A and B are on the left half (3). Shearing reduced linkage between these genes to less than 0.5% of the original value (2). In order to form specific heterologous end-to-end hydrogen-bonded aggregates, MN or control DNA half-molecules (1 μ g/ml final concentration), carrying the i^h and $sus A^+B^+$ genes on their right and left halves, respectively, were mixed with a 20-fold excess of λi^{434} $sus AB$ DNA half-molecules (20 μ g/ml final concentration) in TCM buffer [0.01 M tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.1; 0.01 M $CaCl_2$; 0.01 M $MgCl_2$]. The DNA samples were heated to 76 C, cooled very slowly overnight, and then incubated at 20 C for 3 days. Samples containing MN or control DNA halves annealed with λi^{434} $sus AB$ DNA halves were diluted at least twofold, heated to the temperatures shown, and then chilled rapidly in an ice water-salt mixture. Transformation assays were performed at 30 C for 30 min using cells infected with λi^{434} $sus AB$ helper phage. By using appropriate indicator strains, the i^h and $sus A^+B^+$ activities in each sample were assayed (2). The maximum transformation activities were taken to represent 100% disaggregation. A, Control DNA half-molecules. B, MN-DNA half-molecules. Symbols: —●—●—, i^h , right half-molecule; —○—○—, $sus A^+B^+$, left half-molecule.

DNA concentration. Reconstruction experiments show that the i^h activity of a constant amount of MN-DNA halves was decreased by the addition of increasing amounts of i^{434} $sus AB$ halves.

High DNA concentrations also favor reannealing. The excess normal DNA halves also become available for reannealing after heating above 50 C. MN-altered ends anneal more readily than normal ends (2); therefore, the reannealing effect would be more pronounced with an altered half than with an unaltered one. We believe the loss of i^h activity after heating to temperatures at which normal ends melt is due to a combination of the above factors. Although this precludes quantitation, the qualitative conclusion seems clear. Annealed control or MN-DNA left halves melt near 60 C, whereas MN-DNA right halves annealed with normal left halves melt below 40 C. Therefore, only the right half shows de-

tectable evidence of MN damage. In support of this conclusion, sequence studies utilizing *Escherichia coli* DNA polymerase I catalyzed repair with MN-DNA cohesive ends as templates indicate that the terminal four bases are cleaved from the right 5' end of each molecule while the left end remains intact (4).

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