An Exonuclease Specific for Double Stranded Deoxyribonucleic Acid

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SUMMARY

A nuclease specific for double stranded DNA has been isolated and partially purified from Hemophilus influenzae. The enzyme has a pH optimum of 8.2 to 8.4 and requires a divalent cation, either Mg++ or Mn++. It is most active on DNA whose size has been reduced by either sonic disruption or limited digestion with pancreatic deoxyribonuclease or spleen acid DNase. The enzyme releases only 5'-mononucleotides and leaves a single stranded segment of DNA which is resistant to further hydrolysis. Furthermore, the enzyme destroys the transforming activity of H. influenzae DNA very slowly. These results indicate that the enzyme is an exonuclease, hydrolyzing from the ends of DNA molecules. In addition, the enzyme also exhibits a DNA-phosphatase activity and releases inorganic phosphate from DNA fragments which terminate in 5'-phosphates, suggesting that this enzyme begins hydrolysis from the 3' end of the DNA strands.

The process of bacterial transformation in H. influenzae begins with the irreversible uptake of double stranded DNA by competent bacterial cells and ultimately results in the formation of a heteroduplex chromosomal structure in which one strand comes from the donor DNA and the other from the bacterial chromosome (1).

Notani and Goodgal (1) have shown that while one intact strand of the transforming DNA is inserted into the bacterial chromosome, the other strand appears to be degraded and the donor atoms reincorporated into the DNA. Furthermore, these authors observed that the size of the unintegrated donor DNA is reduced as a function of time after DNA uptake. This finding is a possible explanation for the earlier observation that a fraction of the donor-transforming DNA is inactivated after it is taken up by the bacterial cells (2). Recently Steinhart and Herriott (3) have shown that the addition of transforming DNA to a competent preparation of H. influenzae, whose chromosomes are highly labeled with tritiated thymidine, causes a specific release of radioactive DNA, which is resistant to further hydrolysis. Such a process has been postulated for pneumococcal transformation (4). Furthermore, the DNA-dependent release of radioactive material observed by Steinhart and Herriott (3) might be due to the excision of one strand of the bacterial chromosome by an exonuclease. Therefore, we undertook a study of the nucleases in H. influenzae and attempted to characterize them and correlate their activity with the process of transformation.

This paper reports the isolation and characterization of an exonuclease from H. influenzae. This enzyme is similar in many respects to exonuclease III of Escherichia coli (5) and the pneumococcal exonuclease reported by Lacks and Greenberg (6). The similarity between the H. influenzae enzyme and the pneumococcal exonuclease is especially interesting since not only does bacterial transformation occur in both species, but also the mechanism by which it occurs must be similar since in both species only a single strand of the donor DNA is incorporated into the bacterial chromosome (25).
in distilled water to a final concentration of 20 mmoles of DNA radioactivity from 2P-DNA. The radioactive DNA was diluted twice more with chloroform-octanol.

The DNA in the upper phase was separated from the smaller molecules by passage through a Sephadex G-100 column (2.2 x 30 cm) equilibrated with Buffer A. The fractions containing the DNA were pooled and saved. The final yield of DNA was about 100 μg and contained between 30,000 and 50,000 cpm per μg of DNA.

Nuclease Assay Conditions—The assay used for detection of nuclease activity was based on the production of acid-soluble radioactivity from 3P-DNA. The radioactive DNA was diluted in distilled water to a final concentration of 20 mmoles of DNA nucleotide per ml. When sonicated DNA was used, the DNA was sonicated for 1 min in a 60-watt ultrasonic disintegrator (Measuring and Scientific Equipment, Limited, London). Denatured DNA was prepared by heating the diluted DNA at 100° for 10 min and quickly cooling the heated DNA solution in an ice bath. The final assay solution contained 5 mmoles per ml of DNA nucleotide, 0.1 m Tris HCl, pH 8.2, 0.0025 m MgCl2, 0.001 m DTT in 0.2 mg per ml of BSA (Armour). The enzyme was diluted into 0.02 m Tris-HCl, pH 7.6, 0.001 m DTT in 0.2 mg per ml of BSA before use. The reaction was started by adding 0.3 ml of the diluted enzyme to 3.2 ml of the assay solution above to give a final volume of 3.5 ml or a multiple thereof. All assays were carried out at 37°. The reaction was stopped by adding 1-ml aliquots of the reaction mixture to 0.4 ml of a 15 mg per ml herring sperm DNA (Calbiochem) solution in the cold and adding 0.8 ml of a 5% (w/v) trichloroacetic acid solution. The mixtures were allowed to sit in the cold for 10 min before the precipitates were removed by centrifugation at 10,000 x g for 10 min. Aliquots (1.5 ml) of the supernatant were added to 0.5 ml of a 4 x NH4OH, 0.01% SDS solution on stainless steel planchet, dried, and counted in a windowless gas flow counter.

Preparation of Enzyme—H. influenzae cells were grown in brain heart infusion broth (Difco) supplemented with hemin and NAD (8) with constant aeration at 37°. The cells were harvested in late log phase (A600 = 0.53) by centrifugation. The culture was checked for contamination by streaking the cells on nutrient agar plates containing 5% sheep blood and incubating for 18-24 h. The bacterial extracts which contained large amounts of nucleic acid were prepared by using the procedure described by Ando (12). The specific activity of the enzyme was 2200 μmole/min/μg of protein.

Radioisotopes—Carrier-free 32P-orthophosphate was purchased from E. R. Squibb and Sons, New Brunswick, New Jersey. 1H-DNA isolated from H. influenzae, strain SrNov5, grown in the presence of 3H-thymidine was a generous gift of Mrs. Margaret Robbins.
Nuclease activity on heat-denatured sonically disrupted DNA

The extract was made by suspending 16 g of H. influenzae cells in 32 ml of 0.02 M Tris, pH 7.6-0.05 M NaCl, and passing the suspension through a French pressure cell and centrifuging as described under "Methods." The final supernatant was diluted with 2 volumes (70 ml) of 0.05 M NaCl-0.02 M Tris, pH 7.6, and the nucleic acids precipitated by slowly adding 15 ml of 5% (w/v) streptomycin-sulfate solution. The precipitate was collected by centrifugation and redissolved in 50 ml of 1 M NaCl-0.02 M Tris, pH 7.6. The assay was carried out under the usual conditions at pH 7.6 using heat-denatured sonically disrupted DNA as a substrate.

| Fraction                  | Protein concentration mg/ml | Enzyme units ×10 | Specific activity enzyme units/30 min/mg protein | Yield % |
|---------------------------|-----------------------------|-----------------|-----------------------------------------------|--------|
| Crude extract             | 35                          | 33              | 28.5                                          | 100.0  |
| Streptomycin-sulfate super- | 7.1                         | 12              | 14.8                                          | 48.6   |
| tant.                     |                             |                 |                                               |        |
| Streptomycin-sulfate precipitate | 5.0                        | 16              | 63.5                                          |        |

**TABLE II**

Purification of exonuclease from H. influenzae

All fractions were assayed with sonically disrupted DNA at pH 7.6. The enzyme units recovered in the combined DEAE fractions and (CaPO₄) gel supernatant fractions are corrected for the volume of the extract used in the purification step described under "Methods."

**Fig. 1.** pH optimum of the exonuclease. Sonically disrupted [3P]-DNA was hydrolyzed by 500 units per ml of the purified exonuclease at various pH values in the presence of MgCl₂. The buffer was 0.1 M Tris adjusted with HCl to the pH indicated, and except for the pH of the buffer the standard reaction conditions were used. Four time points were used for each rate determination.

Results

**Purification and Characterization of Enzyme**

Extracts of H. influenzae hydrolyze both sonically disrupted and heat-denatured sonically disrupted DNA as assayed by the conversion of DNA into acid-soluble products (Tables I and II). When streptomycin sulfate is used to precipitate the nucleic acids in these extracts, the nuclease activity which degrades denatured DNA tends to precipitate with the nucleic acids while the activity towards sonically disrupted DNA remains in the supernatant. First attempts at separating the enzyme activity which degrades denatured DNA from the nucleic acids in the streptomycin-sulfate precipitate were unsuccessful, so no further purification of this activity was attempted.

**Table II** gives a summary of the purification of the exonuclease from H. influenzae found in the streptomycin-sulfate supernatant. The specific activity of the enzyme eluted from the calcium-phosphate gel indicates an 85-fold purification over the crude extract. The partially purified enzyme is somewhat unstable at 0-4° and was normally stored frozen at -60°. Storage at 0-4° for 2 weeks resulted in the loss of about 50% of the enzyme activity. Repeated freezing and thawing also inactivated the enzyme.

**Fig. 1** shows pH optimum of the enzyme was about 8.2 to 8.4 under the conditions used in this assay. The enzyme requires a divalent cation, Mg⁺⁺ or Mn⁺⁺. The optimum Mg⁺⁺ ion concentration for these reaction conditions is about 0.0025 M, and if Mg⁺⁺ is omitted or EDTA is added, the rate of the reaction is less than 2% of the rate at optimal Mg⁺⁺ ion concentration. The optimum magnesium ion concentration is the same if native or denatured DNA is used as the substrate; however, the rate of hydrolysis is greatly reduced. Manganese ions substitute for magnesium ions with an optimum concentration of around 0.0006 M Mn⁺⁺. Calcium and zinc ions are not effective.

The enzyme is sensitive to increasing ionic strength. For example, increasing the sodium ion concentration in the assay medium to 0.076 M inhibited the rate by 77%. At a sodium ion concentration of 0.14 M, the inhibition was 91% and at 0.24 M the inhibition was 98%. Potassium ions also inhibited. Likewise, high levels of commercial tRNA inhibited the enzyme.

**Substrate Specificity**

**Fig. 2B** shows that the enzyme hydrolyzes sonicated DNA at a much greater rate than it does native or denatured DNA. In this experiment the rate with native DNA is 4% of that with sonically disrupted DNA, while with denatured DNA the rate is 7.4% of that with sonically disrupted DNA. In five such experiments with the use of different DNA and enzyme preparations, the rate with native DNA averaged 6.8% of that with sonically disrupted DNA, and with denatured DNA the rate was 8.7% of that of sonically disrupted DNA. The higher rate obtained with sonically disrupted DNA indicates the possibility that the enzyme is an exonuclease which hydrolyzes at the end of the DNA.
**Fig. 2.** A, effect of concentration of native DNA on hydrolysis by enzyme. The assay was carried out as described under "Methods" at a pH of 8.2 in the presence of MgCl₂ with an enzyme concentration of 160 units per ml. ○, 80 mmoles per ml; □, 40 mmoles per ml; ●, 20 mmoles per ml of native DNA as nucleotide. The complete hydrolysis of the DNA would be represented by 2100 cpm. The number of counts observed was always greater than 500 for the 30-min sample and greater than 1000 for longer times. B, assay of enzyme activity on native, sonically disrupted, and heat-denatured DNA. The assay was carried out as described under "Methods" at a pH of 8.2 in the presence of MgCl₂ with an enzyme concentration of 160 units per ml. The sonically disrupted DNA and denatured DNA were prepared as described under "Methods." ○, sonically disrupted DNA; □, denatured DNA; ●, native DNA.

**Fig. 3.** Ability of *H. influenzae* exonuclease to hydrolyze pancreatic DNase-treated DNA. ⁴²P-DNA was hydrolyzed with pancreatic DNase at a final concentration of 20 mmoles per ml in 0.002 M MgCl₂-0.02 M Tris, pH 7.0, for 30 min at 30°. The concentration of the enzyme used was 150 μg per ml, 1,500 μg per ml, and 15,000 μg per ml. The reactions were stopped by phenol extraction and dialysis. Samples of the isolated DNA were then treated with 1,000 units per ml of *H. influenzae* exonuclease at pH 8.2 with MgCl₂ and the extent of hydrolysis determined. The minus enzyme controls were DNA samples carried through the incubation and phenol extraction without any added pancreatic DNase. ○, sonically disrupted DNA; ●, Δ, △, native DNA previously treated with DNase, 15,000 μg per ml, 1,500 μg per ml, and 150 μg per ml, respectively; □, no previous treatment with DNase.

of the DNA chain. This conclusion is supported by the fact that the rates of hydrolysis for sonically disrupted DNA and untreated DNA are proportional to the size of the DNA since the sonically disrupted DNA was approximately 30 times smaller than untreated DNA. The activity observed with denatured DNA could be due to a contaminating enzyme.

The hypothesis that this enzyme is an exonuclease is strengthened by the observation that the rate of hydrolysis of native DNA is proportional to the DNA concentration, but the extent of hydrolysis (the fraction of the added DNA converted to acid-soluble products) remains the same. Under the conditions used above for assaying enzyme activity on native DNA, the number of ends of the DNA chains determines the rate (Fig. 2A).

Lacks and Greenberg (6) have isolated an exonuclease which begins hydrolysis at single strand nicks in the DNA chain. Since genetic transformation occurs in both *Pneumococcus* and *H. influenzae*, it is important to determine if the exonuclease from *H. influenzae* will also initiate hydrolysis at single strand nicks. The endonuclease, pancreatic DNase, introduces single strand nicks into DNA. The number of phosphodiester bonds which this enzyme must break in order to reduce the weight average molecular weight by a factor of 2 has been estimated by Thomas (13) to be 200. Therefore, this enzyme is a useful tool for introducing single strand nicks into DNA. *H. influenzae* ⁴²P-DNA was hydrolyzed with 0, 150, 1,500, and 15,000 μg per ml
of pancreatic DNase at 30° for 30 min. The DNA was reisolated by phenol extraction and tested for its ability to act as a substrate for the *H. influenzae* enzyme. Fig. 3 shows that the DNA treated with the higher levels of pancreatic DNase is a good substrate for this enzyme. Fig. 4 shows that this DNA sediments more slowly than control DNA in neutral sucrose gradients. Therefore, it seems likely that the increased activity of this enzyme toward DNase-treated DNA was due primarily to double strand breaks.

**Nature of Products of Reaction**

**Acid-soluble Hydrolysis Products**—Two lines of evidence indicate that the acid-soluble products released by the *H. influenzae* enzyme were 5’-mononucleotides. Fig. 5 shows that the acid-soluble material produced by the *H. influenzae*-catalyzed hydrolysis of sonicated DNA eluted at the same position as 5’-mononucleotide standards when chromatographed on Dowex 50-AG by the method of Blattner and Erickson (14). The recovery of the radioactivity from the column was 95%, and the AT/GC ratio of the recovered material was 1.7 which agrees well with the published AT/GC ratio of 1.63 for *H. influenzae* DNA.

**Table III**

**Action of snake venom 5’-nucleotidase upon sonically disrupted DNA**

The acid-soluble hydrolysis products used in this assay were obtained by hydrolyzing sonically disrupted DNA at pH 7.6 in the presence of MgCl₂ as described in the legend to Fig. 6. After adding carrier DNA, one-half of the reaction mixture was stopped by adding 3% trichloracetic acid, the other by adding 0.6 M HClO₄. After cooling on ice, the precipitates were removed by centrifugation in the cold. The trichloracetic acid was removed by extracting the supernatant 5 times with an equal volume of ether. The HClO₄ supernatant was neutralized to pH 7 with 2 M KOH, cooled in ice, and the resulting precipitate of KClO₄ removed by centrifugation. The snake venom 5’-nucleotidase assay was carried out in a total volume of 0.5 ml containing 0.1 M glycine-NaOH buffer, pH 9.0-0.01 M MgCl₂, and the amounts of the acid supernatants and 5’-nucleotidases indicated. The incubation was for 1 hr at 37°, after which the tubes were chilled and the following added in order: 0.7 ml of H₂O, 0.2 ml of 0.2 M NaH₂PO₄, 0.2 ml of 1 M HCl, and 0.4 ml of a 1% acid-washed Norit suspension in H₂O. The Norit was removed by centrifugation and the supernatants plated and counted.

**Source of acid-soluble hydrolysis products**

| Amount of super- | Amount of 5’-nucleotide | Radioactivity not adsorbed | Amount of super- | Amount of 5’-nucleotide | Radioactivity not adsorbed |
|-----------------|------------------------|--------------------------|-----------------|------------------------|--------------------------|
| Amount tested   | Amount tested          |                          | Amount tested   | Amount tested          |                          |
| ml              | mg                     | %                        | ml              | mg                     | %                        |
| 0.1             | 0                      | 0                        | 0.1             | 0                      | 0                        |
| 0.1             | 0.01                   | 100                      | 0.1             | 0.01                   | 111                      |
| 0.1             | 0.05                   | 94                       | 0.1             | 0.05                   | 100                      |
| 0.2             | 0.01                   | 88                       | 0.2             | 0.01                   | 91                       |
| 0.2             | 0.05                   | 91                       | 0.2             | 0.05                   | 98                       |

**Fig. 5. Separation of acid-soluble products on Dowex 50-AG.**

14P-DNA from *H. influenzae* was sonicated and hydrolyzed by the *H. influenzae* enzyme in the usual manner at pH 7.6 in the presence of MgCl₂ with 1110 units per ml of enzyme. After 1 hour at 37° the reaction was stopped by adding the usual amounts of unlabeled carrier DNA and 0.6 M HClO₄ in place of 5% trichloracetic acid. This solution was chilled in an ice bath, centrifuged, and the supernatant removed. The pH of the supernatant was adjusted to ~3.2 with 2 M KOH in the cold, and the resulting precipitate of KClO₄ was removed by centrifugation. The neutralized supernatant (0.5 ml) was diluted with 0.5 ml of 0.1 M ammonium formate adjusted to pH 3.2 with formic acid, and chromatographed on a Dowex 50-AG column by the method of Blattner and Erickson (14). Samples (0.7 ml) were collected, dried, and counted in a gas flow counter. The arrows indicate the position of 5’-mononucleotide standards prepared and chromatographed as described before (14).
The acid-soluble material was also found to be 5'-mononucleotides by paper chromatography.

The second line of evidence that the acid-soluble products were 5'-mononucleotides came from their susceptibility to snake venom 5'-nucleotidase. This enzyme releases orthophosphate from 5'-mononucleotides but not from 3'-mononucleotides (16). Table III shows that the acid-soluble hydrolysis products are adsorbed to charcoal if no 5'-nucleotidase is added to the reaction mixture. When the acid-soluble hydrolysis products were treated with this enzyme, an average of 95% of the radioactive phosphate was no longer adsorbed to the charcoal. Control experiments were done to prove the specificity of the 5'-nucleotidase which did not release orthophosphate from 3'-nucleotides (<1% of the 5'-nucleotidase activity).

**Acid-insoluble Hydrolysis Products**—The finding that 5'-nucleotides were released from DNA and that sonic disruption of the DNA greatly increased the rate of hydrolysis suggested that this enzyme was an exonuclease acting at the ends of the DNA chains. The following lines of evidence further substantiated this hypothesis.

If the enzyme-catalyzed reaction was allowed to continue to completion, about 50% of the 32P-DNA was resistant to further hydrolysis. Fig. 6 shows that the addition of more enzyme at this point did not greatly enhance the quantity of material solubilized. This experiment was repeated at pH 8.2, in the presence of manganous ions and at lower DNA concentrations, and in each case between 40 and 60% of the DNA remained resistant to further hydrolysis. The reaction did not stop completely, but continued at a much slower rate. This result suggests that the enzyme was behaving similarly to exonucleases which hydrolyze double stranded DNA from one end and leave a single strand fragment which is resistant to further hydrolysis (e.g. exonuclease III of E. coli (5) and the λ-induced exonuclease (17)).

CsCl density gradient centrifugation was used to investigate the nature of the large molecules remaining after nuclease digestion. Because of its small size, sonically disrupted DNA does not band sharply in CsCl, so the experiment was done with native DNA. Native 32P-DNA was hydrolyzed in the presence of manganous ions at pH 8.2 for 5 hours, when 41% of the DNA was acid soluble. Two samples were taken during the course of the hydrolysis.
FIG. 8. Hydrolysis of large molecule products of exonuclease digestion by a nuclease from A. oryzae. Sonicated 32P-DNA was hydrolyzed with 810 units per ml of H. influenzae exonuclease and the extent of hydrolysis determined as usual. At 0 min and 3 hours after addition of the enzyme, 4-ml samples were removed and extracted with an equal volume of phenol saturated with Buffer A. After 3 hours of incubation, 810 units per ml of additional enzyme were added and the incubation continued for 3 more hours when another 4-ml sample was removed and extracted with Buffer A-saturated phenol. After the phenol phases were removed by centrifugation, the aqueous phases were extracted 2 times with an equal volume of ether and dialyzed overnight against Buffer A. Samples of DNA from the dialyzed fraction were treated at 37° with 0.08 μg per ml of the nuclease from A. oryzae prepared according to Ando (12). The incubation mixtures contained 0.033 M sodium acetate buffer, pH 5-3.0, 10⁻⁵ M ZnSO₄, 0.002 M DTT, and 0.5 μg of H. influenzae exonuclease-treated 32P-DNA per ml. Control samples containing 0.5 μg of either sonically disrupted or heat-denatured sonically disrupted DNA were assayed at the same time. ○, denatured DNA; ■, exonuclease-treated DNA (44% hydrolysis); ●, sonically disrupted DNA.

hydrolysis and added to equal volumes of 0.4% Sarkosyl in 0.04 M EDTA, pH 7.4, in the cold. These samples were then banded in CsCl in the Spinco SW-39 rotor and fractions collected and counted. The results given in Fig. 7 show that as hydrolysis proceeded the remaining DNA banded closer to the position of heat-denatured DNA. The 32P-DNA peaks decreased in size and became progressively broader. This was due to the reduction in size of the DNA molecules by hydrolysis. The DNA hydrolyzed for 5 hours banded at the position of denatured DNA.

An exonuclease isolated from A. oryzae by the method of Ando (12) provided a useful tool for investigating the nature of the large molecule products remaining after exonuclease digestion. This enzyme shows a high specificity for single stranded DNA. The rates with which this enzyme hydrolyzed sonically disrupted DNA and heat-denatured DNA are shown in Fig. 8. This figure also shows that the large molecule products of the exonuclease digestion are readily hydrolyzed by the Ando endonuclease. Furthermore, the rate with which the A. oryzae enzyme hydrolyzed the products of the exonuclease digestion increased as the extent of hydrolysis increased. These data confirm the conclusion that the large molecule products remaining after exonuclease digestion are single strands of DNA.

Further evidence that the H. influenzae enzyme is in fact an exonuclease comes from a study of the loss of biological activity with increasing hydrolysis. Richardson, Lehman, and Kornberg (18) have reported that with E. coli exonuclease III after 5% hydrolysis, 50% of the biological activity remains as determined by bacterial transformation, and Lacks and Greenberg (6) have reported that even after extensive digestion (37% hydrolysis) with the pneumococcal exonuclease, 15% of the original biological activity remains. Similarly with the H. influenzae enzyme, as shown in Fig. 9, there is relatively little loss in biological activity as the extent of hydrolysis increases. The relatively low levels of hydrolysis obtained are due to the fact that native DNA was used in this experiment. Sonication destroys almost all of the biological activity (19). These results show that this enzyme is not cleaving the DNA molecule endonucleolytically.
Exonuclease III of E. coli and the pneumococcal exonuclease both show DNA-phosphatase activity (5, 6). These enzymes release inorganic phosphate from DNA fragments which terminate in 3'-phosphomonoesters. Spleen acid DNase and pancreatic DNase are useful tools for producing 3'- and 5'-phosphate-terminated DNA fragments (20, 21). Fig. 10 shows that the H. influenzae exonuclease rapidly releases inorganic phosphate from DNA fragments produced by spleen acid DNase (3'-phosphate terminal fragments) but not from pancreatic DNase-produced fragments (5'-terminal phosphates). There is a very slow linear release of inorganic phosphate from 5'-phosphate-terminated fragments which might be a result of a contaminating nucleotidase or phosphatase. The total amount of inorganic phosphate released from 3'-phosphate-terminated DNA is 75% of the inorganic phosphate released by alkaline phosphatase. This failure to obtain a complete release of inorganic phosphate might be due to the presence of either internal phosphomonoesters or terminal 5'-phosphomonoesters.

**DISCUSSION**

The finding that the H. influenzae enzyme hydrolyzes sonically disrupted DNA at a much greater rate than it does intact DNA first suggested that the ends of the DNA molecule were the initial site of hydrolysis and that this enzyme was an exonuclease. This assumption was strengthened by the finding that the acid-soluble products released are 5'-mononucleotides. The fact that the enzyme rapidly hydrolyzes about 50% of the DNA and that further hydrolysis occurs at only about 5% of the initial rate was explained by the finding that the acid-insoluble product remaining is single stranded DNA. These results show the enzyme is in fact an exonuclease and hydrolyzes DNA molecules in a stepwise manner from the ends of the molecule until single strand molecules are formed. The finding that this enzyme possesses a DNA-phosphatase activity specific for DNA fragments terminating in 3'-phosphates further suggests that the initial site of hydrolysis occurs at the 3' ends of the DNA chains. If a 3'-phosphate is present, it is released as inorganic phosphate before 5'-mononucleotides are released.

As Lack (22) has pointed out, the data on the inactivation of the transforming activity by this enzyme should be viewed with caution. It is possible that the enzyme is selectively hydrolyzing small DNA molecules which do not transform despite the fact an excess of enzyme is used in these experiments. Alternatively, the DNA molecules which do transform may be resistant to hydrolyzing by the exonuclease.

The mechanism by which this enzyme hydrolyzes DNA is similar to that of exonuclease III of E. coli (5), an exonuclease from Pneumococcus (6), and an exonuclease from Bacillus subtilis (23). It is interesting to note that bacterial transformation is a well-known phenomenon in H. influenzae, Pneumococcus, and B. subtilis, and there has recently appeared a report that transformation also occurs in E. coli (24). While it is possible that this coincidence has nothing to do with the process of transformation per se, it is interesting to see how such an enzyme might play a role in this process.

In both H. influenzae and pneumococcal transformation, only one strand of the donor-transforming DNA is incorporated into the bacterial chromosome (1, 25). In Pneumococcus this is explained by the finding that the donor DNA is hydrolyzed to mononucleotides and single stranded DNA (4) soon after the DNA is bound to the competent cells. The situation is different in H. influenzae in that no single stranded DNA can be found during transformation despite the fact only a single strand is ultimately incorporated into the chromosome (1). However, it could be that integration occurs so fast in this species that no pool
of single stranded material can accumulate. An enzyme such as this exonuclease is a likely candidate for the production of single stranded material. These enzymes could, of course, play another role in the transformation process. For example, they could excise one strand of the bacterial chromosome to prepare a region of DNA for the integration of the donor-transforming DNA strand.

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