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# SPECIFICITY OF DNA UPTAKE IN GENETIC TRANSFORMATION OF GONOCOCCI

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Summary. Genetic transformation of gonococci to streptomycin resistance was inhibited by homologous DNA or by DNA from related Neisseriae, but not by high concentrations of heterologous DNAs. Gonococci were capable of adsorbing large quantities (up to about 50 μg per $10^8$ cells) of both homologous and heterologous DNA, which could not be eluted by strong shearing forces. Treatment with externally added DNase removed virtually all the heterologous DNA while a small fraction of the homologous DNA, not influenced by the presence of excess heterologous DNA, remained cell-bound in a form resistant to nuclease treatment. Competing homologous DNA suppressed nuclease-resistant binding. These findings suggest that gonococci have two types of DNA binding components at their surface. Competence of gonococci for genetic transformation undergoes a rapid decay if the cells are incubated with homologous (but not with heterologous) DNA.

INTRODUCTION. Genetic transformation of bacteria is initiated by the capture and adsorption of exogenous DNA molecules to binding components at the cell surface. Recent experiments have revealed a high degree of specificity in this adsorption step. In competent pneumococci the DNA binding sites seem to be restricted to double stranded polydeoxynucleotides, double stranded polyribonucleotides or double stranded polyribo-polydeoxyribonucleotide hetero duplexes do not compete for these binding sites (4,12). An even higher degree of specificity was observed in the Hemophylus transformation system in which uptake of exogenous DNA was restricted to homologous DNA or DNA isolated from taxonomically related species (7). A recent, elegant investigation by H. Smith, Sisco and Deich has produced evidence indicating that the basis of this selectivity is the recognition of a specific base sequence in the exogenous DNA by a surface located bacterial protein (3). We describe here still another type of interaction between exogenous DNA and competent gonococci which appear to have two kinds of DNA binding components.

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Materials and Methods. 1) Bacterial strains: Neisseria gonorrhoeae strain 51 colony type T2 and T4 were provided by Dr. Richard B. Roberts of Cornell University Medical College. A streptomycin resistant ($STR^R$) strain (M.I.C. > 1 mg/ml) of this organism was constructed by transformation. N. gonorrhoeae MHD 316 (Hypoxanthine$^-$, Proline$^-$, Uracil$^-$, Arginine$^-$) was obtained from Dr. Wesley Catlin, Medical College of Wisconsin, Milwaukee, Wisconsin. N. meningitidis was obtained from Dr. Emil Gotschlich of this university. N. subflava (ATCC 10555) was from the American Type Culture Collection. Bacteria were maintained by daily transfer on solid medium in candle jars at 37°C.

2) Medium The liquid medium used in this study was Brain Heart Infusion (DIFCO) with 1% defined supplements (Isovitalex) and 4 mM $MgCl_2$. For agar plates, GCBA Agar (Baltimore Biological Laboratories) with defined supplements was used.

3) Transformation Procedure The procedure for obtaining competent cells was essentially that used by Sarubbi et al. (6). Clonal types T2 were scraped from an 18 hr GCBA plate and suspended in prewarmed BHI-supplements-$Mg^{++}$. The cells were routinely suspended to a Nephelos value between 100 and 200 using a Coleman Nepho-Colorimeter. A value of N = 100 represents approximately 1.5 X $10^8$ colony forming units. One milliliter of cells was added to tubes containing the DNA and incubated at 37°C for 20 minutes. The reaction was terminated with 50 µg/ml pancreatic DNase (Worthington 2X crystallized) and incubated 5 mins at 37°C. The cells were then diluted ten and one hundred-fold in Brain Heart Infusion and 0.1 ml of each dilution added to 4 ml of soft GCBA agar at 48°C, mixed and immediately layered onto the surface of plates containing 20 ml of GCBA agar plus supplement. The plates were incubated at 37°C for at least 7 hours to allow phenotypic expression and then each plate received 5 mls of soft GCBA agar containing sufficient streptomycin to give a final concentration of 300 µg/ml throughout the plate. After 4 days of incubation, colonies were counted with a New Brunswick colony counter.

4) DNA Binding and Uptake Assay Cells from agar plates were suspended (cell concentration: 3 X $10^8$ viable units per ml) in Beef Heart Infusion with supplements and 4 mM $MgCl_2$. Radioactively labeled DNA was added to 2 mls of this suspension and incubated for 20 minutes. For total binding, the cells were chilled and washed by centrifugation (12,000 X G, 10 mins, 4°C) 3 times with ice cold medium. The pellet was resuspended in cold saline and an aliquot transferred to tubes containing ice cold 10% $CCl_3COOH$ (2 mls). After 10 mins on ice, the precipitate was collected on Whatman GF/A glass fiber filters, washed 2X with cold 10% $CCl_3COOH$ and 2X with 95% ethanol. Radioactivity was assayed by placing the discs in 10 mls of toluene based scintillation fluid using a Nuclear-Chicago Mark II scintillation spectrometer. For DNase resistant uptake, the cells were incubated with labeled DNA for 20 mins and then received 50 µg/ml DNase for 5 mins at 37°C. The cells were then chilled and processed as described above for total binding.

5) DNA preparations. E. coli B, Cl. perfringens, M. luteus DNAs were obtained from Sigma Chemical Co. λ viral DNA was from Miles Laboratories. All were dissolved in saline. DNA was extracted from Neisseria species and Str. pneumoniae by sodium dodecyl sulfate-deoxycholate lysis of the cells (5). The DNA was suspended in 150 mM NaCl-15 mM sodium citrate and dialyzed at 4°C against 2 changes of the same buffer (500 volumes each) and 2 changes of saline 260 nm in a Zeiss PMQ-2 spectrophotometer where one absorbance unit = 50 µg DNA.

Radioactive DNA was obtained by biosynthetic labeling (8). 3H-pneumococcal DNA was from Str. pneumonial TVS (thymidine requirer) labeled with $^3H$- thymidine (56 Ci/m mol New England Nuclear) in CpH8. Specific activity of the DNA preparation was 2.6 X $10^6$ CPM/µg. N. gonorrhoeae MHD 316 was labeled with $^3H$ uracil (23.3 Ci/m mol- New England Nuclear) in Beef Heart Infusion plus supplements. Specific activity of this DNA preparation was 1.43 X $10^5$ CPM/µg.

For testing the shear-sensitivity of DNA adsorbed to the cell surface, the cells were vortexed on a Vortex Jr. Mixer (maximum speed setting) for 5 seconds after each resuspension in the washing procedure. Control cells were gently

TABLE 1

EFFECT OF HETEROLOGOUS DNA PREPARATIONS ON THE GENETIC TRANSFORMATION OF GONOCOCCI

| Source of Transforming DNA | Source of Competing DNA | Number of Transformants (X $10^5$) per ml (Concentration of competing DNA µg per ml) 0 | 5 | 10 | 50 | 100 |
|---|---|---|---|---|---|---|
| N. gonorrhoeae[a] | none | 1.94 | --- | --- | --- | --- |
| | E. coli B | --- | 3.01 | 3.11 | 2.30 | 1.76 |
| | Cl. perfringens | --- | 4.3 | 3.22 | 2.32 | 1.90 |
| | M. luteus | --- | 2.90 | 2.50 | 2.60 | 2.40 |
| | Str. pneumoniae | --- | 3.26 | 3.0 | 2.30 | 2.30 |
| | Phage λ | --- | 2.18 | 1.81 | 1.99 | 1.68 |
| | N. gonorrheae | --- | 0.8 | 0.5 | 0.13 | 0.08 |

[a] Homologous, $STR^R$ DNA (with and without competing DNas) was added at a concentration of 1 µg/ml and the transformation assays performed as described under Methods.

resuspended by finger agitation. This vortexing would remove surface-bound DNA in the pneumococcal transformation system.

Results. Effect of heterologous and homologous DNA on the genetic transformation of gonococci. Competent gonococci were exposed to mixtures of transforming gonococcal DNA (carrying the streptomycin resistance marker; DNA concentration: 1 µg/ml, a concentration sufficient to saturate the transformation assay, see Fig. 2) and various heterologous DNAs at different concentrations and the ability of these competing DNAs to suppress the frequency of transformation was tested. Table 1 shows that none of the six heterologous DNA preparations had marked inhibitory effect on the transformation frequency even when used at 100-fold excess concentration. A substantial increase in the number of transformants was noted at low concentrations of competing heterologous DNA; the reason for this effect is not understood at the present time.

In contrast, Figure 1 demonstrates that homologous DNA and DNA isolated from two Neisseria taxonomically related to gonococci significantly inhibited

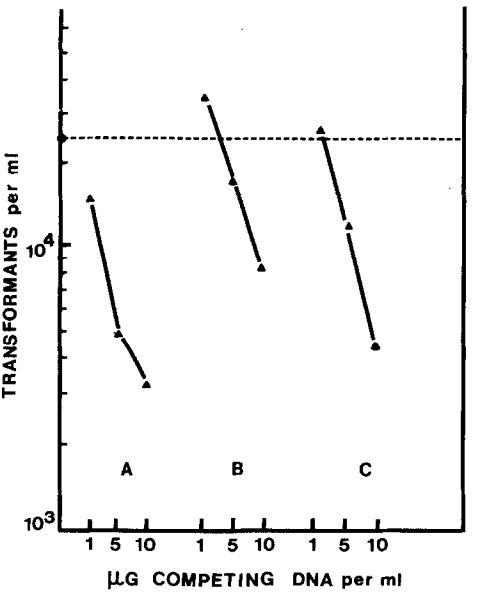



Figure 1. Competition of DNA from related Neisseria species.

DNA was purified from N. subflava and N. meningitidis, as well as from $Str^S$ gonococci. 1 ml of T2 Gonococci (N = 105) were added to tubes containing:

A. 1 µg $Str^R$ Gonococcal DNA and 1, 5 and 10 µgs of $Str^S$ gonococcal DNA
B. 1 µg $Str^R$ Gonococcal DNA and 1, 5 and 10 µgs of $Str^S$ N. subflava DNA
C. 1 µg $Str^R$ Gonococcal DNA and 1, 5 and 10 µgs of $Str^S$ N. meningitidis DNA.

Cells were assayed for $Str^R$ transformants after 20 minutes of incubation at 37°C. Reaction was terminated with 50 µg/ml DNase.

gonococcal transformation and the degree of inhibition was proportional to the concentration of the competing DNAs.

Specificity of DNA uptake Table 2 demonstrates that competent gonococci can adsorb substantial quantities of both homologous and heterologous DNA but only homologous DNA becomes bound in a form resistant to treatment with externally added pancreatic DNase, presumably due to cellular uptake. While competing, non-radioactive DNA suppresses the amounts of surface-adsorbed DNA (both homologous and heterologous), only homologous competing DNA (i.e. DNA from gonococci) causes inhibition of nuclease-resistant DNA binding. When cells that had DNA bound were subjected to shearing forces, there was no

TABLE 2

BINDING AND UPTAKE OF DNA BY COMPETENT GONOCOCCI

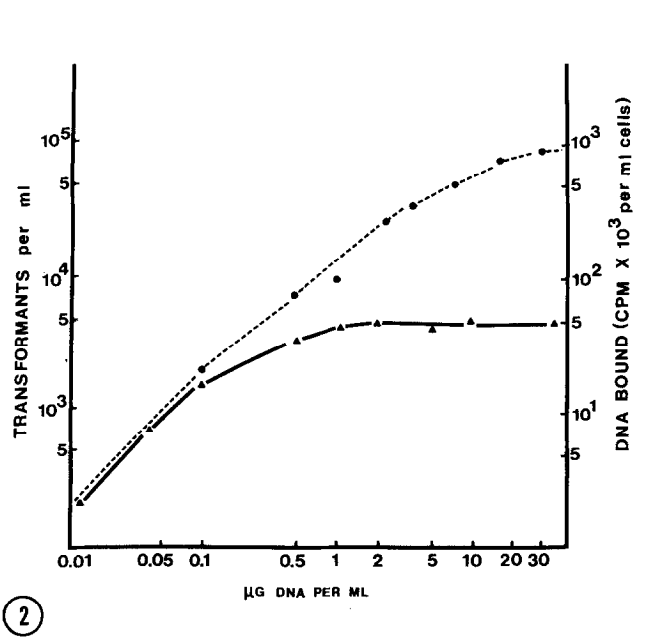
| Radioactive DNA (µg/ml) | Competing DNA (µg/ml) | DNA Binding (Cell-associated DNA, ng per 2 X $10^8$ cells) | DNA Uptake (nuclease resistant binding, ng per 2 X $10^8$ cells) |
|---|---|---|---|
| pneumococcal (1.0 µg) | none | 45 | 0.16* |
| | gonococcal (10) | 26.5 | 0.32 |
| | pneumococcal (10) | 22.3 | 0.20 |
| gonococcal (1.0 µg) | none | 65.7 | 4.1 |
| | gonococcal (5 ) | 34.1 | 2.1 |
| | pneumococcal (5 ) | 28.1 | 3.8 |
| | E. coli (5 ) | 21.6 | 4.3 |

*less than 1% DNase resistant binding represents background.

substantial loss of counts as compared to a gently resuspended control (19852 CPM vortexed sample; 20568 CPM control sample). The same results were obtained using T4 colony type cells.

Saturation of DNA binding sites Figure 2 shows that gonococci are capable of binding relatively large amounts of DNA (as measured by the adsorption of radioactive DNA). At cell concentrations of 3 X $10^8$ viable bacteria per ml, saturation of this binding capacity occured at about 50 µg/ml pneumococcal DNA. At the same cell concentration, a maximum level of genetic transformation was attained by 1 µg DNA per ml.

Decay of gonococcal competence to undergo genetic transformation Portions of a gonococcal culture were preincubated with homologous $STR^S$ DNA, homologous $STR^R$ DNA was added at intervals and the number of $STR^R$ transformants was determined (Fig. 3). A decline in $STR^R$ transformants occurred with in-



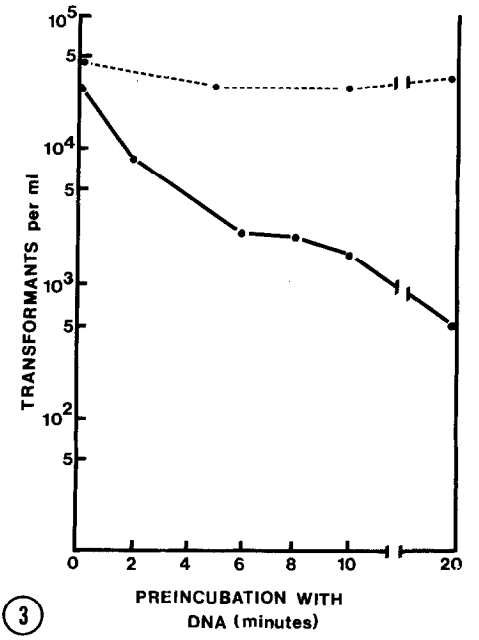




Figure 2. Saturation of Binding and Transformation in Competant Gonococci

Total binding of increasing concentrations of radioactive pneumococcal DNA (2.26 X $10^6$ CPM/µg) to 2 mls of T2 Gonococci (N = 180) was measured as described under Methods (●---●). For transformation, increasing concentrations of $Str^R$ gonococcal DNA were added to (N = 165) $Str^S$ T2 Gonococci and the number of transformants to streptomycin resistance was assayed (▲——▲). All incubations were for 20 minutes at 37°C.

Figure 3. Decay of Competence by Pretreatment with Homologous DNA.

Cultures of $Str^S$ Gonococci (N = 150) received 1 µg homologous $Str^S$ DNA at time 0. At intervals, 1 µg of homologous $Str^R$ DNA was added and the number of transformants after 25 minutes of incubation at 37°C was assayed (●——●). For comparison purposes, 1 µg of pneumococcal DNA was added to 1 ml of competent cells, and 1 µg of $Str^R$ gonococcal DNA added at indicated intervals and assayed for transformants (●- -●). All incubations were terminated with 50 µg/ml DNase.

creasing time of preincubation in $STR^S$ DNA. Preincubation with the heterologous $STR^S$ DNA did not cause a similar loss of competence.

Discussion. The observations reported here suggest that the surface of competent gonococci contains 2 independent kinds of DNA binding sites or receptors. These two receptors have several contrasting features. i) Both homologous and heterologous DNA adsorb to receptor 1, while binding to receptor 2 seems to be

restricted to homologous DNA or DNA isolated from taxonomically related species. ii) Large quantities of DNA can adsorb to gonococci via receptor 1 but most, if not all, of these molecules remain at a site accessible to external DNase, while a portion of DNA attached to receptor 2 (amounting to 4 ng gonococcal DNA per 2 X $10^8$ cells or about 6% of the DNA adsorbed to the cells at saturating DNA concentration) becomes resistant to DNase treatment, presumably because of intracellular transport. There appeared to be no loss of DNA from the surface when the cells were subjected to shearing forces (vortexing) that remove DNA from pneumococcal cells (9). This indicates that the DNA is protected in some fashion. The role of pili in shear protection is ruled out by the finding that bound DNA is shear resistant in both T2 and T4 (non-piliated) cells. iii) Heterologous DNA in the medium or attached to receptor 1 has virtually no effect on the uptake of homologous DNA or on the frequency of genetic transformation. In contrast, homologous DNA molecules competes for receptor 2. iv) Maximum amounts of DNA can bind to receptors 1 even at low temperature (0°C), while receptor 2 seems to function only at physiological temperatures since both DNA uptake and transformation are inhibited at 0°C (unpublished observations). v) A further evidence for the specificity of receptor 2 for homologous DNA molecules is illustrated by the finding (Fig. 3) that rapid decline of gonococcal transformability may be initiated by incubation with homologous (but <u>not</u> with heterologous) DNA. The mechanism of this phenomenon is not understood at the present time.

Genetic transformation of gonococci has been first described by Sparling (10) and further studies have revealed several interesting features of DNA uptake in this bacterium. It was found that only colonial types 1 and 2 have high levels of transformability (10) while all the four colonial types were capable of DNA binding (2) and it has been suggested that the surface appendages (pili) of T1 and T2 cells may play a role in DNA uptake (1). Several studies have dealt with physiological parameters of gonococcal transformation (11).

The novelty of the observations described in this report is the apparent specificity of gonococcal DNA uptake system for homologous molecules. In this report, gonococcal transformation seems to resemble closely the mechanism of genetic transformation in Hemophylus influenzae (3,7). In the first detailed description of the specificity of the Hemophylus system, Scocca and his associates considered either the activity of a restriction enzyme or a base-sequence specificity of the DNA receptors as the possible molecular basis of selective DNA uptake (3). Recent studies of H. Smith and his colleagues indicate that the latter is the case (3, 9 ).

The specificity of gonococcal DNA uptake described in this report suggests that acquisition of genetic elements from heterologous sources via genetic transformation may not occur in gonococci growing in their natural environment.

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Literature cited

1. Biswas, G.D., Sox, T., Blackman, E. and Sparling, P.F. (1977). J. Bacteriol. 129, 983-992.
2. Catlin, B.W. (1967). J. Bacteriol. 94, 719-733.
3. Deich, R.A., Sisco, K. and Smith, H.O.(1978). p. 46 Abstracts of the 4th European Meeting on Bacterial Transformation and Transfection, University of York, York England.
4. Lacks, S. (1977). p. 35-44 in Portoles, A., Lopez, R. and Espinosa, M. (eds.), Modern Trends in Bacterial Transformation and Transfection, North-Holland Press, Amsterdam.
5. Marmur, J. (1961). J. Mol. Biol. 3, 208-218.
6. Sarubbi, F.A. and Sparling, P.F. (1974). J. Inf. Dis. 130, 660-663.
7. Scocca, J.J., Poland, R.L. and Zoon, K.C. (1974). J. Bacteriol. 118, 369-373.
8. Seto, H. and Tomasz, A. (1974). Proc. Nat. Acad. Sci. US 71, 1493-1498.
9. Sisco, K. and Smith, H.O. (1979) Proc. Natl. Acad. Sci. US, in press.
10. Sparling, P.F. (1966). J. Bacteriol. 92, 1364-1371.
11. Sparling, P.F., Biswas, G.D. and Sox, T.E. (1977). p. 155-176 in Roberts, R.B. (ed.) The Gonococcus, J. Wiley and Sons, New York.
12. Tomasz, A. (1973). p. 81-88 in Archer, L.J. (ed.) Bacterial Transformation, Academic Press, New York.