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Transformation in Pneumococcus: Existence and Properties of a Complex Involving Donor Deoxyribonucleate Single Strands in Eclipse

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Donor deoxyribonucleic acid (DNA) single strands exist in a complex during the eclipse phase in pneumococcal transformation. This eclipse complex exhibited specific physical properties distinct from those of both pure DNA single strands and native DNA. These included a lower affinity for diethylaminoethyl-cellulose and hydroxylapatite than that of single-strand DNA, faster sedimentation than the DNA chains that it contains, and a buoyant density in Cs_2SO_4 lower than that of native DNA. The complex was dissociated by treatments with sodium dodecyl sulfate, NaOH, guanidine-hydrochloride, chloroform, and proteinase K but was insensitive to ribonuclease.

The genetic recombination that occurs during deoxyribonucleic acid (DNA)-mediated transformation of pneumococcus (*Streptococcus pneumoniae*) produces a recombinant chromosome almost entirely of recipient origin, but in which small segments of single chains have been replaced by homologous segments of donor DNA containing 3 to 9 kilobases (9, 13, 12). This recombination process now appears to occur in several discernible stages. First, native donor DNA bound to the competent cell surface is cut, through both strands, to yield double-strand fragments of about 3 to 9 kilobases (18, 22). Second, single chains from these fragments are taken into the cell (15), with concomitant release to the medium of an equal mass of oligonucleotides (16, 18, 21, 22). Finally, some of the single-strand fragments in the cell are degraded, whereas others engage in an "attack" on the chromosome, displacing homologous resident strand segments (17, 21). Thus, the length of the final integrated piece is, at least partly, determined extracellularly; that it is just a single strand is determined by the uptake mechanism; and the intracellular event(s) comprises a displacement of a resident strand segment by a single-strand fragment of donor DNA. DNA extracted while in the single-strand stage exhibits very little transforming activity for donor genetic markers (8), primarily because it is single stranded (10, 11, 20, 26), and it has therefore been described as being "in eclipse" (7, 14). During recovery from eclipse, donor marker transforming activity observed on assay of lysates increases as integration into the recipient genome proceeds (8, 10).

Searching for a convenient method by which to measure the progress of the integration reaction, we examined the behavior of ^{3}H-labeled donor DNA, after uptake by competent cells, on columns of hydroxylapatite. The results revealed that single strands in eclipse exist in the form of a complex, which is specifically characteristic of intracellular single strands and whose properties might be relevant to understanding uptake, eclipse, and integration. In this paper we report experiments demonstrating the existence of the complex and its specificity for intracellular single strands.

MATERIALS AND METHODS

Bacterial strains. DNA was prepared from strain CP1014, carrying an erythromycin resistance marker from strain 5 MC (1, 27) and a set of mutations conferring a requirement for 0.75 μg of thymidine per ml. The recipient (CP1015) carried the streptomycin resistance marker of 5 MC. Both strains of *S. pneumoniae* were derived from strain Rx-1 (1).

Culture media. Basal medium (CAT) containing Casitone, tryptone, yeast extract (all Difco), NaCl, glucose, and phosphate buffer was prepared as described previously (21), except that either enzymatic casein hydrolysate (Nutritional Biochemicals Corp.) was substituted for Casitone or the medium was filtered (Millipore Corp.; HAWP, 0.45-μm pore size) and then autoclaved twice to reduce particulate content and persistent sporeforming contaminants to an acceptable level.

Sugar-phosphate-albumin solution was as described by Lacks and Greenberg (16), but with glucose as the sugar.

Materials. Chemicals and materials other than standard commercial reagents were obtained as

follows: proteinase K and pancreatic deoxyribonuclease I (DNase) were from Boehringer-Mannheim; [5-*methyl*-^{3}H]thymidine and [2-^{14}C]thymidine were from Schwarz/Mann; Pronase and bovine pancreatic ribonuclease (RNase) were from Calbiochem; 6-(*p*-hydroxyphenylazo)-uracil was a gift from B. W. Langley; hydroxylapatite was prepared according to Tiselius et al. (28) and was a gift from M. Curiale; diethylaminoethyl (DEAE)-cellulose and 2% agarose gel beads were from Bio-Rad Laboratories; 8% agarose gel beads were from P-L Biochemicals; bovine serum albumin, fraction V, was from Sigma Chemical Co.

Transformation assays. Assay of competence and of yields of erythromycin-resistant transformants was done as described (21), except that the plates were made by the immediate double-overlay method, to be described elsewhere.

DNA preparation. For isolation of labeled DNA, strain CP1014 was grown for several generations in basal medium supplemented with [^{14}C]thymidine (52 mCi/mmol, 0.1 μCi/ml) or [^{3}H]thymidine (45 Ci/mmol, 100 μCi/ml) to about 4×10^8 colony-forming units/ml, chilled, washed, and lysed as described previously (21). For [^{14}C]DNA, the lysate was treated with RNase, then deproteinized with chloroform, sheared in the cold by repeated passage through a 26-gauge hypodermic needle, and dialyzed against SSC (0.15 M NaCl-0.015 M sodium citrate, pH 7). For [^{3}H]DNA, the crude lysate was treated with RNase and then with Pronase and purified by chromatography at 22°C on an 8% agarose column.

Transformation. Competent cultures were prepared by a procedure incorporating features of procedures described previously (2, 9, 19, 24). A stock of cells grown in CAT to about 2×10^8 colony-forming units/ml and frozen was thawed, diluted 1:100 in CAT supplemented with 0.2% bovine serum albumin and 1 mM $CaCl_2$, and incubated at 37°C for about 90 min, and then the entire culture was frozen in 30- or 60-ml portions. For use, frozen tubes were thawed in an ice-water bath, centrifuged at 8,000 $\times$ *g* at 0 to 4°C for 6 min, resuspended in 1/10 volume of the supernatant, and incubated for about 10 min at 37°C for completion of development of competence. The optimum times of incubation before freezing competent cells, and after concentrating thawed competent cultures, were determined for each batch of CAT medium and of stock cells. To allow substantial uptake of donor DNA with a minimum of integration into the recipient chromosome, these concentrated competent cells were chilled to 25°C, and DNA was added, followed 3 to 10 min later by DNase (50 μg/ml). After 30 s of further incubation, the culture was chilled and washed three times with SSC in the refrigerated centrifuge. For the experiments of Fig. 1 to 4, these transformed cells were immediately suspended in cold lysis buffer [0.05 M tris(hydroxymethyl)aminomethane (Tris), pH 8.0-0.03 M ethylenediaminetetraacetate (EDTA)-0.4% Sarkosyl-0.1% Triton X-100], and lysed by warming to 37°C for 3 min. For the other experiments, cells were exposed to DNA in the presence of 100 μM 6-(*p*-hydroxyphenylazo)-uracil to reduce DNA synthesis during DNA uptake, and the transformed cells were washed and finally resuspended in sugar-phosphate-albumin plus 10% glycerol and frozen in 1- or 2-ml portions for storage at −82°C. For use, one tube was thawed at 0°C, warmed to 37°C if required, and then chilled, supplemented with 0.1 volume each of 0.3 M EDTA and 1% Triton X-100, and lysed, as above, at 37°C.

In all these steps, cultures to be frozen were supplemented with sterile glycerol to 10% (by volume), frozen by immersion in a bath of dry ice and acetone, and stored in a freezer at −82°C.

Hydroxylapatite chromatography. Columns were constructed from 3-ml polypropylene syringe barrels. A small (0.7-ml) bed of hydroxylapatite was supported, and covered, by porous (25-μm pore size) polyethylene disks. The columns were washed with 0.02 M phosphate buffer. All elutions were performed with sodium phosphate buffers adjusted to pH 6.8. Each sample was diluted to 0.02 M phosphate buffer and passed through a column, to be collected as fraction 1. Succeeding 1-ml fractions were collected as the column was eluted with increasing concentrations of phosphate buffer. A wash of 0.02 M phosphate buffer was followed by a linear concentration gradient of phosphate buffer at room temperature unless otherwise indicated. The volume of the wash, and the volume and initial and final concentrations of the gradient, are indicated in individual figures. A set of up to 12 parallel columns was operated by means of a peristaltic proportioning pump distributing buffer from a single reservoir or mixing chamber. All panels of a given figure represent columns eluted in parallel at one time. Fractions were collected for 3.3-min intervals at a flow rate of about 0.30 ml/min per column directly into scintillation vials, as described below, except that only 1 ml of fraction 1 was counted. During elution, the total liquid volume in each column was maintained at 1 to 1.5 ml.

DEAE-cellulose chromatography. A small column with a 1-ml bed of Cellex-D (Bio-Rad) was prepared and operated as described for hydroxylapatite chromatography, but at 4°C. The column was washed with, and the sample diluted in, 10 mM NaCl-10 mM Tris (pH 8)-1 mM EDTA. Subsequent elution was performed with the same buffer containing increasing concentrations of NaCl, as described in the legend for Fig. 7.

Agarose chromatography. Columns of agarose 1.5 cm in diameter were prepared with bed volumes of 30 to 50 ml. Each was calibrated by measuring the elution volumes for native DNA and thymidine. Columns were equilibrated with the running buffer and eluted, after application of 0.5 to 2 ml of sample made 5% in glucose, at a rate of 0.35 ml/min. One-tenth or one-twentieth of each fraction was taken for determination of radioactivity.

Determination of radioactivity. Fractions from equilibrium gradients in CsCl or Cs_2SO_4 were collected on filter-paper strips, dried, washed in 10 and 5% trichloroacetic acid at 0°C and then with acetone, and dried. Paper sections were immersed in toluene containing 5 g of 2,5-diphenyloxazole per liter for counting in a Packard liquid scintillation spectrometer. Other aqueous samples were mixed

with 9 volumes of scintillation fluid containing 500 ml of Triton X-100 and 5 g of 2,5-diphenyloxazole per liter of toluene, plus sufficient water prevent formation of precipitates or visible separation of phases. The counting efficiency was shown, by use of an internal standard of [^{3}H]thymidine, to be constant for all fractions of a typical hydroxylapatite column. The figures present data as total observed count rate corrected for isotope spillover only.

RESULTS

Chromatography of DNA in eclipse. A competent pneumococcal culture was exposed to ^{3}H-labeled DNA at 25°C for a period short enough that essentially all donor DNA taken up would be still in eclipse. These transformed cells were then treated with DNase, chilled, washed, and incubated with Triton X-100 to induce autolysis. Part of the clear lysate was mixed with denatured [^{14}C]DNA and chromatographed on a small column of hydroxylapatite. Figure 1 shows that donor DNA label eluted as a single peak at a lower concentration of phosphate buffer than the denatured DNA used as a standard. The remainder of the lysate was divided, and portions were subjected to various treatments before chromatography on a series of parallel hydroxylapatite columns. The data of Fig. 2 show that after treatment with concentrated guanidine-HCl, the donor DNA label elutes at the phosphate concentration characteristic of single strands and that Pronase digestion causes a partial shift in the same direction, but that 0.35 phosphate buffer does not. Similar experiments (data not shown) showed that chloroform or phenol treatment also cause a shift to the characteristic single-strand elution pattern.

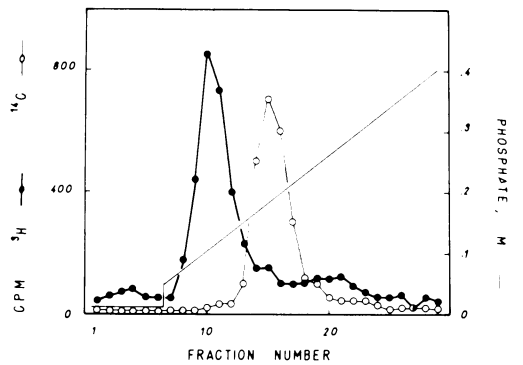

FIG. 1. *Hydroxylapatite chromatography of donor DNA in a lysate of competent cells immediately after uptake. Competent cells were exposed to [^{3}H]DNA for 5 min and then treated with DNase, chilled, washed, frozen at −82°C, thawed at 0°C, washed in SSC, and lysed in the presence of 0.4% sodium lauroyl sarkosinate. A 30-μl amount of lysate and 10 μl of denatured ^{14}C-labeled DNA were diluted in 2 ml of 0.01 M phosphate buffer and passed over a hydroxylapatite column; the column was washed with 2.5 ml of 0.01 M phosphate buffer and eluted with a linear 0.05 to 0.4 M gradient of phosphate buffer.*

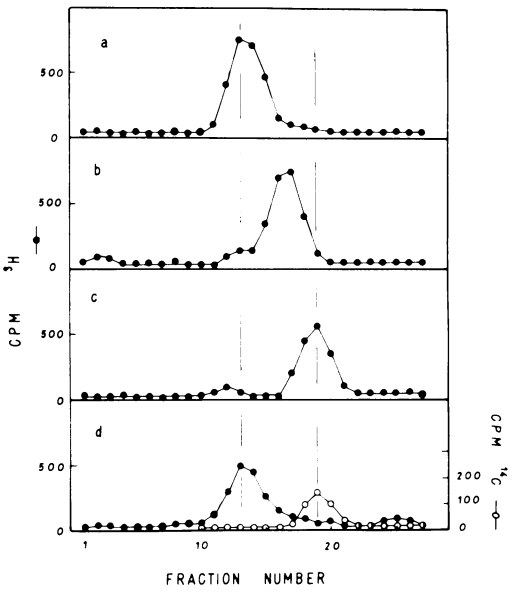

FIG. 2. *Effects of guanidine-HCl and Pronase on donor DNA elution profile. Portions (20 μl) of the lysate of transformed cells used in Fig. 1 (after 20 h at 0°C) were incubated in 200-μl volumes with (a) 0.35 M phosphate buffer, (b) Pronase at 500 μg/ml, or (c) 6 M guanidine-HCl, at 22°C for 60 min. (d) Denatured [^{14}C]DNA was added to an untreated portion kept at 0°C as a control. Each was then diluted with 2 ml of 0.01 M phosphate buffer, and hydroxylapatite chromatography was carried out with elution by a gradient of 0.05 to 0.35 M phosphate buffer following 4 ml of 0.01 M phosphate buffer.*

These results suggested that donor DNA strands exist in such lysates in a complex that alters or blocks the characteristic adsorption of DNA to hydroxylapatite.

Specificity of the complex for intracellular DNA. Since it is possible that such a complex might form nonspecifically between single strands and cell components in a lysate, either after lysis or at some specific stage during lysis, a control experiment was performed to detect such an association, even if it were limited to the small amounts of single-strand DNA present in lysates of transformed cells. Competent cells identical to those of Fig. 2 were treated as before, but donor DNA was omitted. Instead, samples of the same [^{3}H]DNA used as donor for Fig. 1 were sheared, denatured, and added to the washed cells in lysis

buffer before, or after, lysis, in amounts similar to those present in lysates of transformed cells. Figure 3 shows that both the single strands present during lysis and those added to the crude lysate eluted from hydroxylapatite at the same phosphate concentration as the denatured [^{14}C]DNA standard.

Thus, the property of early elution is specifically characteristic of intracellular single strands.

Hydroxylapatite chromatography (as in Fig. 1) of lysates of samples of a culture of transformed cells taken after various times of incubation at 37°C after DNase treatment showed that as donor DNA was integrated into the recipient chromosome the amount of early-eluting label decreased, but its chromatographic properties remained unchanged (data not shown).

Inasmuch as this observed early elution from hydroxylapatite is neither a general effect of components of the lysis buffer nor an effect of components of a crude lysate of competent cells, and is exhibited by donor single strands throughout the eclipse period but not by single strands added during or after lysis, we propose

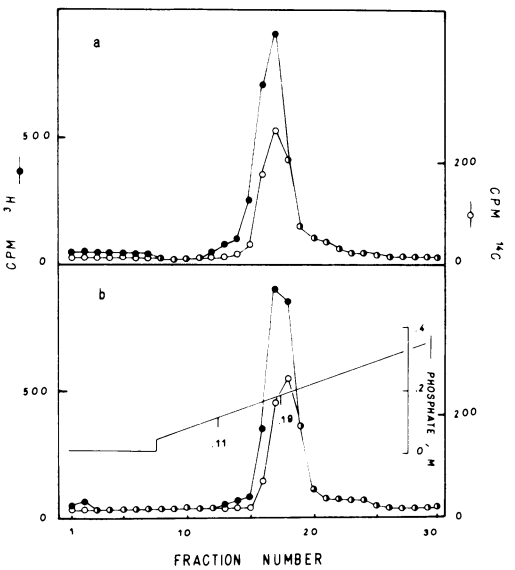

FIG. 3. *Hydroxylapatite chromatography of denatured DNA added to lysates of competent cells. A frozen tube of competent cells from the same batch as used in Fig. 1 was thawed and treated as in Fig. 1, except that no DNA was added before DNase treatment. Instead, a sample of the ^{3}H-labeled DNA was sheared, denatured, and added to one portion (a) of the cells in lysing buffer before lysis and to the other portion (b) after lysis. Denatured [^{14}C]DNA was added to both lysates before hydroxylapatite chromatography.*

to term the structure(s) containing donor single strands during eclipse the "eclipse complex." The list of agents that serve to dissociate this complex suggests at least some protein components.

Purification by agarose gel exclusion chromatography. In pursuit of a method for separating the eclipse complex from most other cell proteins, a lysate of cells transformed with [^{3}H]DNA was treated with RNase and subjected to gel exclusion chromatography on a column of 8% agarose gel. The majority of the donor DNA ^{3}H label emerged at the void volume, well separated from nearly all cell protein and ribonucleic acid, as measured by ultraviolet absorbance (data not shown). The fractions representing this first peak were pooled and analyzed by hydroxylapatite chromatography in parallel with a reserved portion of the crude lysate. About 80% of the agarose-purified donor ^{3}H eluted later than the eclipse complex of the crude lysate, at a position expected for free single strands (data not shown). Agarose chromatography carried out in the cold accomplished a similar purification of eclipse complex with no apparent dissociation to free single strands (Fig. 4).

Dissociation of purified eclipse complex. Eclipse complex purified by hydroxylapatite chromatography was treated with a variety of agents before dilution and rechromatography (data not shown; chromatography as in Fig. 2). The results show that, by this measure, the complex is stable for at least 8 min at 37°C, is degraded by DNase I to a product eluting at about 0.07 M phosphate buffer, and releases a product with affinity for hydroxylapatite nearly identical to that of single-strand DNA on treatment with proteinase K, chloroform, sodium dodecyl sulfate, and NaOH.

Sedimentation velocity. Generation of free single strands with base allowed comparison of the sedimentation rate of eclipse complex with that of the DNA strands contained therein (Fig. 5). Eclipse complex sedimented in neutral sucrose gradients exhibited a heterogeneous distribution with a median rate about 60% greater than that of base-released strands from the same lysate.

Isopycnic centrifugation. Eclipse complex was separated from the bulk of the cell proteins by chromatography on a 2% agarose column, divided into two portions, and centrifuged to equilibrium in CsCl or Cs_2SO_4 solutions, along with a density position marker of ^{14}C-labeled pneumococcal DNA (Fig. 6). Recovery of ^{3}H label from the CsCl gradient was only 10%; from the Cs_2SO_4, it was 50%. The small portion recovered from CsCl banded at the density of

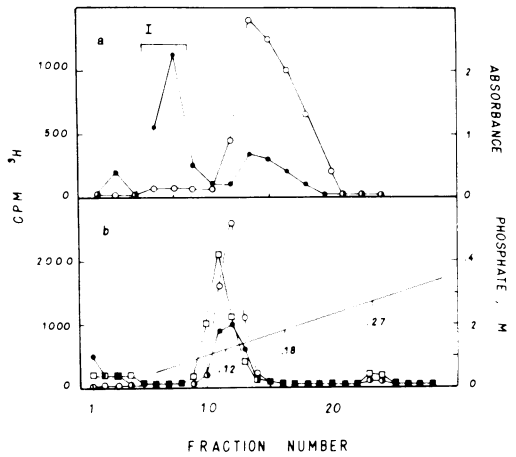

FIG. 4. *Agarose chromatography of eclipse complex at 4°C. A competent culture was exposed to [³H]DNA for 4 min, treated wtih DNase, washed, and lysed at 37°C. A portion of the lysate was treated at 25°C for 10 min with 50 μg of RNase per ml. Part of the RNase-treated lysate was applied to a column of 8% agarose and eluted with 0.05 M phosphate buffer at 4°C. The absorbance at 260 nm (○) was determined for each (2-ml) fraction; a 200-μl volume was taken for measurement of radioactivity (●), and the remainder was stored on ice until pooled as indicated by the bracket in (a). Donor ³H label obtained as pool I from the agarose column was compared to the crude lysate with or without RNase digestion by chromatography on hydroxylapatite. (b) Parallel hydroxylapatite chromatograms of the crude lysate (□), the RNase-treated lysate (○), and pool I (●) from (a). The nominal phosphate gradient is indicated.*

native DNA, whereas the larger portion recovered from Cs_2SO_4 formed a peak less dense than native DNA, with a broad "shoulder" of even lower density.

DEAE-cellulose chromatography. Eclipse complex eluted from a DEAE-cellulose column in two portions (Fig. 7a): form I (65%) eluted in a low-salt buffer; form II (35%) eluted as a single peak at about 0.4 M NaCl. Rechromatography of form II with denatured [^{14}C]DNA showed that form II elutes from this adsorbent just before denatured DNA (Fig. 7b). Forms I and II were then compared by hydroxylapatite chromatography (data not shown). Both eluted in the phosphate concentration region characteristic of eclipse complex (about 0.1 to 0.13 M); but form I emerged as a single peak at about 0.09 M phosphate buffer, whereas form II emerged as two overlapping peaks at about 0.09 and 0.12 M.

DISCUSSION

The stage of pneumococcal transformation between uptake of donor DNA and its incorporation into the cell genome has been termed the "eclipse" (7, 10, 14). The term is based on the low relative transforming activity of genetic markers carried in DNA in this phase (8, 10). Biochemical analysis of DNA in eclipse has shown both that it is single stranded and that it has a shorter chain length than donor DNA (15, 17, 21). The former property can probably account for the low transforming activity in eclipse, since transforming activity in pneumococcus depends strongly on the strandedness (11, 20, 26) of DNA assayed.

The data of the present paper show that when the deproteinizing steps used in the studies cited above are omitted, examination of crude lysates reveals that donor DNA strands in eclipse exist in a complex with additional unknown components, a complex that exhibits specific properties distinct from those of single DNA chains. This complex, termed the eclipse complex, can be dissociated by typical deproteinization treatments, after which donor DNA

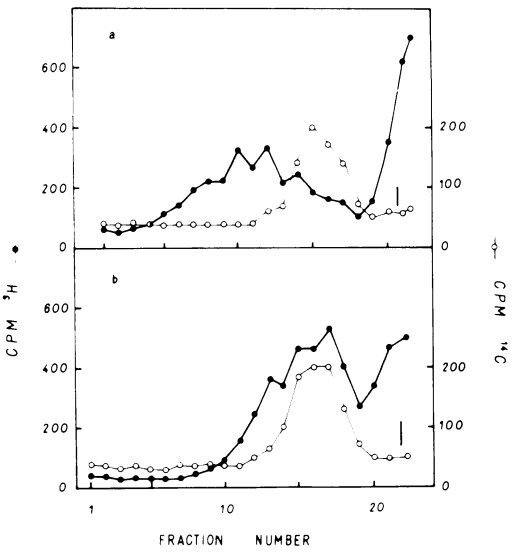

FIG. 5. *Zone sedimentation of intact and dissociated eclipse complex. Frozen transformed cells were thawed and sedimented at 0°C, resuspended in 0.05 M Tris-0.01 M EDTA-0.1% Triton X-100, and warmed for lysis. One half of the lysate (a) was kept at 0°C; the other (b) was mixed with 1/10 volume of 1 M NaOH and neutralized with 1/5 volume 0.5 M NaH_2PO_4 after 5 min at 20°C. Each sample was chilled, mixed with a denatured sheared [^{14}C]DNA reference, layered onto a 5 to 20% sucrose gradient (0.05 M NaCl-0.02 M Tris-0.001 M EDTA, pH 8) prepared in the cold, and centrifuged at 55,000 rpm for 90 min in an SW65 rotor at 10°C. Constant-volume fractions were collected from the bottom of each tube. Bar indicates the position of the center of the applied sample.*

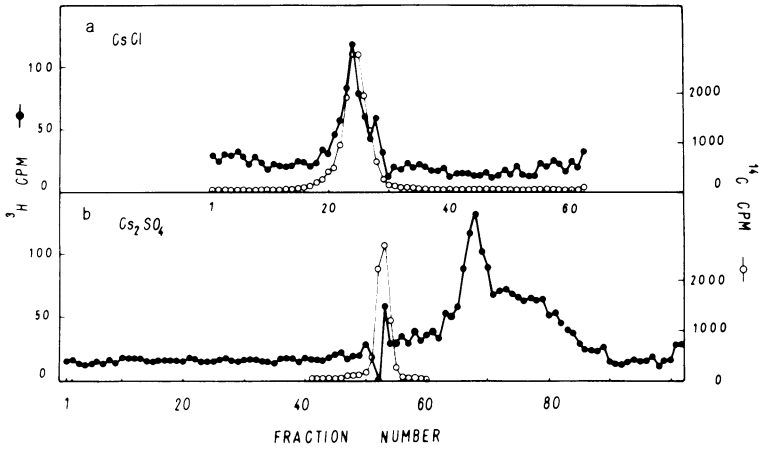

FIG. 6. *Isopycnic centrifugation of eclipse complex. A lysate of transformed cells, prepared as described in Fig. 5, was partially purified by chromatography on a column of 2% agarose in 0.1 M NaCl–0.001 M EDTA–0.01 Tris (pH 8) at 4°C. The peak fractions emerging at the excluded volume were pooled; one half was made 56% CsCl (volume, 2.5 ml), and the other was mixed with 0.67 volume of saturated Cs_2SO_4 (volume, 3.6 ml). Both were centrifuged at 35,000 rpm for 70 h in an SW56 rotor at 10°C. Two-drop fractions were collected from the bottom of each gradient, except that the first 10 fractions of (a) contained 4 drops each, and were assayed for ^3H (●) and the ^{14}C label of native reference pneumococcal DNA (○) included in each tube.*

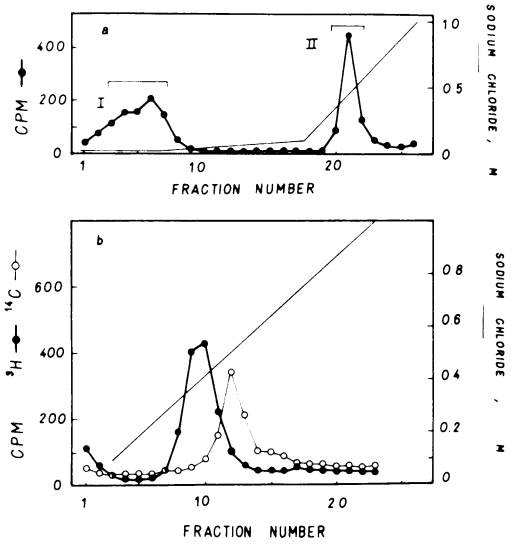

FIG. 7. *Chromatography of eclipse complex on DEAE-cellulose. (a) A lysate of transformed cells prepared as described in Fig. 5 was diluted fivefold in 0.01 M NaCl and passed through a bed of DEAE-cellulose. After elution with 0.01 M NaCl and two successive linear gradients (one, 0.01 to 0.10 M NaCl; the next, 0.1 to 1.0 M NaCl), fractions were pooled as indicated by brackets I and II. (b) A portion of pool II was rechromatographed on DEAE-cellulose with a ^{14}C-labeled denatured DNA standard.*

label exhibits properties characteristic of single-strand DNA.

The property by means of which the eclipse complex was first detected, and with which the existence of the complex was monitored in later experiments, was an unusual behavior in hydroxylapatite chromatography. Specifically, eclipse complex was eluted at a lower phosphate concentration (0.1 to 0.13 M) than single strands of purified DNA (0.17 to 0.18 M) or native DNA (0.25 to 0.28 M). Such a lowered affinity for hydroxylapatite could be due to a specific alteration of the molecular conformation of the DNA chain so that exposed portions of the DNA chain bind less tightly, or it might reflect the affinity of some other complex component(s) for hydroxylapatite, the DNA itself being completely buried in the complex. The observation of two forms of eclipse complex, each with a lower affinity for DEAE-cellulose than that of single-strand DNA, is, of course, consistent with both of these interpretations. The observed increase in sedimentation coefficient and decrease in buoyant density are also to be expected of a complex with components less dense than DNA, but carry no specific implications for its structure.

No direct evidence is presented here on the nature of any non-DNA components(s) of the eclipse complex. That at least one protein is an essential component is strongly suggested by the sensitivity to Pronase and proteinase K; a protein component(s) could also be the basis of the sensitivity to chloroform and sodium dodecyl sulfate and elevated pH. Portions of the data presented here also suggest that there are at least two different forms of eclipse complex. First, the Cs_2SO_4 density gradient results are

consistent with two components, one represented by the sharp peak and the other by the broad peak apparently superimposed on the first. Second, some dissociating treatments appeared to yield two products: about 60% free single strands and 40% of a product eluting from hydroxylapatite at about 0.08 M phosphate. Finally, chromatography on DEAE-cellulose revealed two clearly distinguishable components, each with a specific behavior on hydroxylapatite. Further work will be required to understand the basis of these divisions and the relationships among them.

Comparison of Fig. 1 and 3 with Fig. 4 shows that a substantial minority of donor ^{3}H label inside cells after 4 to 6 min of uptake at 25°C elutes from 8% agarose gel columns later than the eclipse complex emerging at the excluded volume; yet no comparable amount of label elutes from hydroxylapatite columns at a position distinct from that of eclipse complex. We conclude that there is apparently a minor component of the eclipse complex peak with a distinctly lower molecular weight. This component is also apparently visible in the sucrose gradients (Fig. 5) of a crude lysate where total radioactivity in each fraction was measured, but would not be visible in the equilibrium density gradients, which followed a preliminary fractionation on agarose. This minor component may also be represented by one of the species separated by chromatography on DEAE-cellulose. It may well contain the 20% of donor counts found to be acid soluble after uptake at 25°C in previous work (21). These relationships are under further study.

Since the minute amount of single-stranded donor DNA present in a lysate of transformed cells might bind after lysis to one or more of the components present in a crude cell lysate, a reconstruction experiment was performed which showed that similar amounts of single-stranded DNA added to a lysate of competent cells prepared under parallel conditions exhibited the ordinary pattern of elution from hydroxylapatite expected for single strands.

DNA-containing complexes have been observed in lysates of transformed cells in several transformation systems. In *Bacillus subtilis* transformation, whose mechanism is clearly very similar to that of pneumococcus (4, 5), Piechowska and Fox (23) found donor single strands in lysates of transformed *B. subtilis* cells bound in a complex whose density in CsCl was lower than that of free single strands by a variable amount, and which was dissociable by 4 M NaCl at 70°C and treatment with Pronase. Reconstruction experiments showed that similar complexes were found that included denatured (but not native) DNA added to lysates, especially if present during lysis. Any specificity for intracellular DNA was thus uncertain. Davidoff-Abelson and Dubnau (3) later reported that the cell lysis and DNA extraction procedure of Piechowska and Fox, using high lysozyme concentrations at low temperatures, was particularly effective in protecting donor single strands from a degradation to lower molecular weight otherwise seen. They also presented evidence indicating that other basic proteins could substitute for lysozyme in affording such protection and suggested that the complex seen by Piechowska and Fox could be due specifically to the lysozyme used at high concentrations. A single-strand-specific DNA-binding protein apparently specific to competent cells of *B. subtilis* has been reported (6). Raina and Ravin (25) have described donor DNA during eclipse in *S. sanguis* as involved in a complex that is of low buoyant density, resistant to two DNases, and rapidly sedimenting. It may be very similar to that described here. They have more recently shown that the donor DNA in the complex is single stranded (personal communication). Ephrussi-Taylor reported (7) an observation, at some moment early in eclipse in pneumococcus, of a portion of the donor DNA ^{32}P label forming a band at a density of about 1.64 g/cm^{3} in a CsCl gradient. Such material of specific low density may also be related to the eclipse complex described here.

A feature of work with eclipse complex that we do not yet understand is an occasional tendency for donor label in eclipse complex to be unrecoverable. Occasionally, for example, 90% of the activity applied to a hydroxylapatite column does not emerge. That label does not appear in early fractions implies that nuclease digestion of the DNA can be ruled out. It appears that some sort of agglutination or strong affinity for glass or plastic is developed. Treatments that occasionally precede this behavior include proteinase digestion and freezing. The low recovery from the CsCl gradient of Fig. 6a may reflect the same behavior.

The variety of specific chromatographic properties exhibited by eclipse complex and its stability through successive purifications suggest that quite pure complex may be obtainable, albeit in small quantities, for study of non-DNA components.

Although speculation on the details of the mechanism would certainly be premature at this point, the non-DNA components of the eclipse complex(es) described here must surely be prime candidates for roles in promoting and completing recombination. The position of donor DNA in eclipse, both as a product of uptake

and as the precursor for recombination, immediately suggests three possible roles for eclipse complex. First, as a DNA-binding structure, it could promote the progressive transfer of a DNA strand into the cell. Second, it could protect the DNA after uptake from degradation by endo- or exonucleases. Third, it could promote integration, as by, for example, holding bases exposed for hydrogen bonding with temporarily exposed bases of the chromosome.

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