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# PRESYNAPTIC DONOR DNA-PROTEIN COMPLEXES IN TRANSFORMATION OF STREPTOCOCCUS SANGUIS: IDENTIFICATION OF THE PROTEIN COMPONENT

Jawahar L. Raina and Arnold W. Ravin

Department of Biology, The University of Chicago
Chicago, Illinois 60637

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SUMMARY: Complexes containing single-stranded donor DNA, produced after initial binding of native DNA to competent S. sanguis cells and prior to chromosomal synapsis, have been purified, labelled with [$^{125}$I] and shown to possess a unique polypeptide about 15,500 daltons in size.

## INTRODUCTION:

Upon binding of native donor DNA to competent Bacillus subtilis (1,2) and Streptococcus pneumoniae (3) and sanguis (4,5), only a single DNA strand survives and does so complexed to protein and resistant to exogenous DNase I. In S. sanguis such DNA-protein complexes are initially located on or external to the cell membrane, and hence are called presynaptic. The constituent DNA of the presynaptic complex is accessible to degradation by exogenous micrococcal nuclease until translocation to the chromosome internal to the cell membrane (6). This translocation is specifically inhibited by ethidium bromide, which apparently alters the structural integrity of the presynaptic complex, and makes some of the constituent DNA available to degradation by cellular nucleases in vivo (6).

We report here that the protein component of the presynaptic complex of S. sanguis contains a single polypeptide about 15,500 daltons in size.

## METHODS:

The strains of Streptococcus sanguis; preparation of [$^{3}$H]-labelled and unlabelled donor DNA; development of competence; transformation; analysis of lysates of transformed cells in preparative sucrose and CsCl[1] gradients; and sedimentation analysis of pooled fractions in 5-20% neutral sucrose gradients have all been previously described (4,5,6).

[1] Abbreviations used: CsCl, cesium chloride; DNase, pancreatic deoxyribonuclease I; SDS; sodium dodecyl sulfate; TCA, trichloracetic acid.

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For [$^{125}$I]-labelling, an amount of presynaptic complexes containing approximately 1-2 µg of unlabelled donor DNA equivalents was mixed with Borate buffer, pH 8.1 (100 mM), NaCl (0.1 M), Lactoperoxidase (0.3 units), [$^{125}$I]-NaI (0.2 mci), and 30% $H_2O_2$ diluted 1/1000 (3 µl) in a final volume of 0.5 ml. The mixture was incubated at room temperature for 15 min and then returned to ice.

Prior to electrophoresis, [$^{125}$I]-labelled and [$^{3}$H]- thymidine-labelled presynaptic complexes were precipitated with cold TCA (10%), washed twice with cold ethanol and dried in a vacuum oven at 60°C for 30 min. Samples and protein standards were dissolved in 50 µl of denaturing buffer (0.25 M Tris-HCl, pH 6.0; 8% SDS; 4% mercaptoethanol; 15% glycerol), heated at 100°C for 2 min, and subjected to electrophoresis on 10% SDS-polyacrylamide slab gels (1.5 mm thick) prepared according to the method of Laemmli (8). Gels were stained with Coomassie blue (0.25% w/v) and destained in a mixture of methanol-acetic acid-water (5:1:5).

[$^{125}$I]-NaI (∿17 ci/mg) was purchased from New England Nuclear and methyl-[$^{3}$H]-thymidine (50-60 ci/mmole) was a product of Schwarz/Mann; Lactoperoxidase was a product of Calbiochem.

RESULTS:

Purification of presynaptic complexes: Competent S. sanguis cells were exposed for 1 min to [$^{3}$H]-thymidine-labelled or unlabelled donor DNA, DNased, washed, lysed and the presynaptic donor DNA complexes recovered as a slowly sedimenting fraction distinct from the more rapidly sedimenting recipient chromosomal DNA in 10-30% neutral-high salt gradients (4,5). After extensive dialysis (10 mM Tris-HCl, pH 8.1; 10 mM EDTA), the DNA complexes were further purified by banding in CsCl gradients. The density profile of [$^{3}$H]-labelled presynaptic complexes is shown in Fig. 1. The peak density of [$^{3}$H]-label is

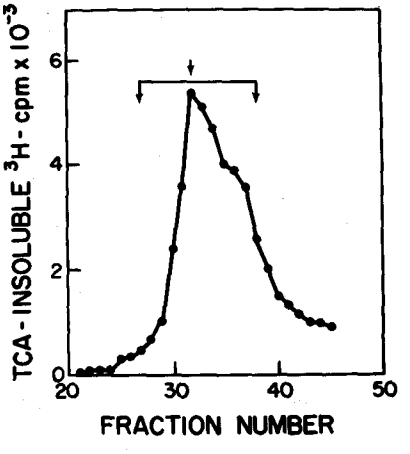


Figure 1. CsCl density profile of [$^{3}$H]-thymidine labelled presynaptic complexes. Presynaptic complexes (∿2 µg donor DNA equivalents), either [$^{3}$H]-thymidine labelled or unlabelled, recovered in the slowly sedimenting fraction after sedimentation of lysed spheroplasts in 10-30% neutral sucrose - high salt gradients were banded in CsCl as previously described (4,5 ). ↓ indicates the position of recipient DNA in a similar gradient.

lighter than that of denatured S. sanguis DNA (not shown) and a major portion bands in the form of a trail of decreasing density. This trailing is characteristic of such complexes, and is probably due to heterogeneity in protein to DNA ratio of the complexes.

Sedimentation analysis: Pooled fractions (indicated by arrows in Fig. 1) from gradients containing [$^{3}$H]-labelled complexes and corresponding fractions from gradients containing unlabelled complexes were dialysed and concentrated to a final volume of about 0.5 ml. Unlabelled presynaptic complexes were then labelled with [$^{125}$I] in vitro. [$^{125}$I]-labelled and [$^{3}$H]-labelled presynaptic complexes were divided into two equal portions: one was shaken gently with phenol (redistilled phenol saturated with Tris-HCl, pH 10; 1 M NaCl; 1% SDS) while the other was held on ice. The phenol-untreated and -treated samples were analysed by zone sedimentation in 5-20% sucrose gradients. Sedimentation profiles are presented in Fig. 2. [$^{3}$H]-labelled DNA in presynaptic complexes sediments faster (35S) than that from similar complexes after deproteinization with phenol (25S, Fig. 2A,B). The molecular weight of single-stranded DNA released upon deproteinization corresponds to about 1.5 megadaltons. Dissociation causes no loss in DNA content as indicated by similar recoveries of the label from the respective gradients. The [$^{125}$I] peak observed in presynaptic complexes labelled with [$^{125}$I] (Fig. 2C) corresponds in sedimentation position to the [$^{3}$H] peak in non-iodinated, [$^{3}$H]-labelled complexes (Fig. 2A), a result to be expected if [$^{125}$I]-labelled protein forms a part of the presynaptic complexes. Deproteinization with phenol, which dissociates the protein in these complexes, should be expected to result in disappearance of the iodine peak (Fig. 2D). This expectation is confirmed by the loss of [$^{125}$I] counts in the position where [$^{3}$H]-labelled DNA sediments upon deproteinization of the complexes with phenol (Fig. 2B). The considerable amount of TCA-insoluble, [$^{125}$I]-label present toward the top of the gradients (Fig. 2C,D) represents non-specific proteins, especially [$^{125}$I]-labelled lactoperoxidase, which is a component of the iodination reaction.

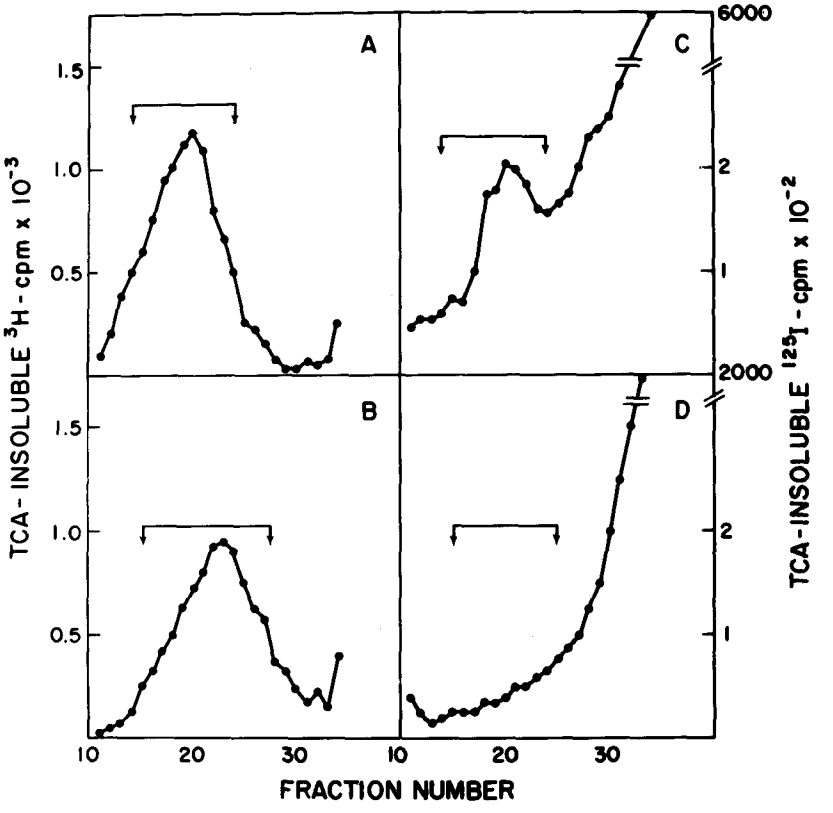


Figure 2. Zone sedimentation analysis of iodinated, non-tritiated and tritiated, non-iodinated complexes. [$^{3}$H]-labelled and unlabelled complexes (fractions indicated by arrows in Fig. 1, and corresponding ones from gradients containing unlabelled presynaptic complexes) were dialyzed extensively against buffer (10 mM Tris-HCl, pH 8.1, 1 mM EDTA) and concentrated to a final volume of about 0.5 ml. Unlabelled complexes were then iodinated with [$^{125}$I] as described in Methods. [$^{3}$H]- and [$^{125}$I]-labelled complexes were subjected to 5-20% sucrose gradient analysis as previously described (5). [$^{3}$H]-labelled presynaptic complexes before (a), after (b) and [$^{125}$I]-labelled complexes before (C), after (D) phenol deproteinization.

Autoradiography: TCA precipitates from pooled fractions (indicated by arrows in Fig. 2A-D) were subjected to electrophoresis on SDS-polyacrylamide gels. Fig. 3 shows an autoradiogram of the dried gel. In Lane 1 (phenol-untreated, [$^{125}$I]-labelled fractions) may be seen a single band of radioactive iodine migrating at a rate intermediate between those of the two reference proteins, ß-lactoglobulin and lysozyme, with respective molecular weights of 18,300 and 14,400. The relative molecular weight of the [$^{125}$I]-labelled polypeptide is estimated to be about 15,500 daltons.

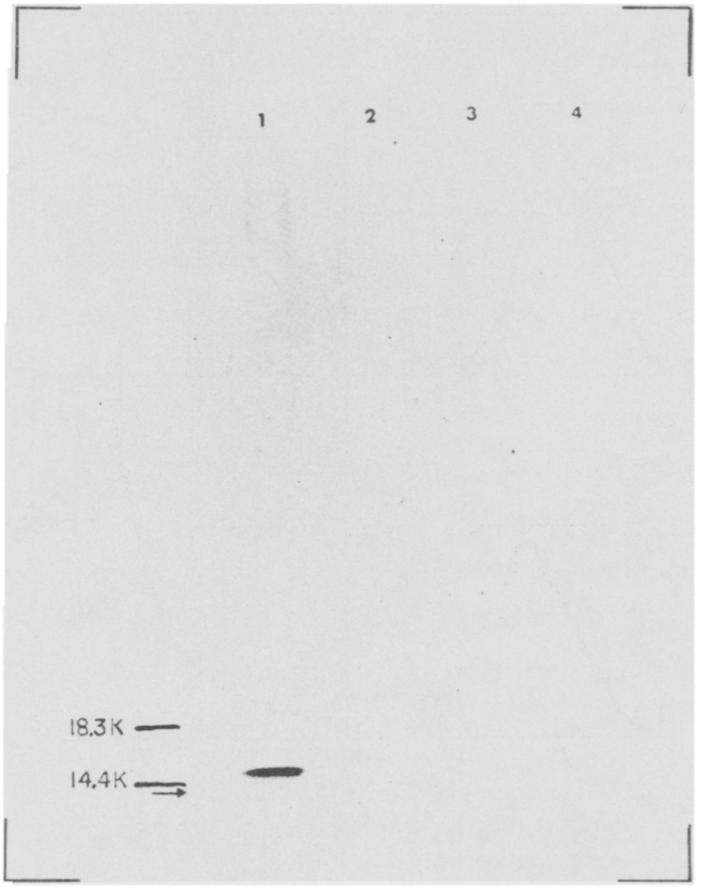


Figure 3: Identification of [$^{125}$I]-labelled protein by autoradiography. [$^{125}$I]- and [$^{3}$H]-labelled complexes (pooled fractions from sucrose gradients indicated by arrows in Fig. 2) were subjected to electrophoresis on 10% SDS-polyacrylamide slab gel followed by autoradiography of the dried gel as described in Methods. [$^{125}$I]-labelled sample before (lane 1), after (lane 2) and [$^{3}$H]-labelled samples before (lane 3), after (lane 4) phenol deproteinization. $^{14}$C-labelled ink was used to indicate the position of standard reference proteins (β-lactoglobulin 18,300 daltons) and lysozyme (14,400 daltons), in the gel. → marks the position of bromophenol blue tracking dye front.

Loss of [$^{125}$I]-label upon deproteinization with phenol is also reflected in a corresponding lack of radioiodine image (Fig. 3, Lane 2), indicating that, although the presynaptic complexes are resistant to high salt concentrations and sarcosyl in the gradients, denaturation with phenol irreversibly dissociates the complex.

DISCUSSION:

We have demonstrated that donor DNA upon uptake by competent S. sanguis recipient cells forms a complex with cellular protein. The iodine peak of [$^{125}$I]-labelled complexes purified from lysates of cells briefly exposed to donor DNA (Fig. 2C) corresponds in position in a sedimentation gradient to that of non-iodinated complexes containing tritiated donor DNA (Fig. 2A). Its position is determined solely by a stable, non-covalent association and, hence, co-sedimentation with donor DNA, since dissociation with phenol separates the iodine label from the DNA (Fig. 2D).

The protein component of the complex appears to be a unique species, since only a single band is observed in an autoradiogram of a denaturing gel on which the iodinated material has been run. The protein is either a polypeptide about 15,500 daltons in size or some multimer of this polypeptide.

In other work we are examining the function of this protein in transformation. We know that it serves to protect donor DNA against inactivation by recipient nucleases (6), and it may have other functions as well in effecting translocation to, synapsis and recombination with the recipient chromosome. Our recent data (manuscript in preparation) indicate that the protein in the presynaptic complex is one of several major proteins induced early during the onset of competence, which is marked by significant transcriptional and translational changes. The inducibility of a whole new set of proteins, concomitant with ability of cells to bind exogenous DNA and transform, has also been observed in Streptococcus pneumoniae (7). One of these pneumococcal proteins (19,500 daltons) binds to single-stranded donor DNA during the "eclipse" phase of transformation, and may play a similar role to that of the protein in the presynaptic complex of S. sanguis.

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