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Purification and Properties of Streptococcal Competence Factor Isolated from Chemically Defined Medium

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A procedure for the isolation and purification of competence factor produced in a defined medium by group H streptococci, strain Challis-6, is presented. Partial characterization and chemical analysis of the product are described. The procedure yields competence factor of high purity, as shown by homogeneity in electrofocusing, by electrophoresis in sodium dodecyl sulfate polyacrylamide gels, and by chemical analysis. The data indicate that competence factor is a small, dialyzable, highly basic compound. It is free from lipids, phosphorus, and carbohydrates, and is colorless and thermoresistant. Its biological activity is destroyed by trypsin but not by deoxyribonuclease, ribonuclease, lipase, or lysozyme. Its high isoelectric point of above *p*H 11.0 suggests that competence factor may be a protamine or a polymer of basic amino acids. The possibility that a polyamine may be an integral part of the polypeptide molecule has not been excluded.

Group H streptococcus strain Challis produces exocellular competence-inducing factors (CF) in complex media (16, 18) and in defined media (9). The addition of CF to cells of the nontransformable *Streptococcus* H strain Wicky makes them competent, as detected by their subsequent transformation to dihydrostreptomycin resistance. This induction of noncompetent cells into the competent state requires protein synthesis for its realization (9, 15, 22). CF that acts similarly has been demonstrated conclusively in only one other species of bacteria, namely, in *Diplococcus pneumoniae* (24). The requirement for protein synthesis by these competence factors distinguishes them from other competence factors described (21). Although streptococcal and pneumococcal competence factors have been studied intensively, their chemical nature and modes of action are not known (21).

We recently reported CF production by noncompetent Challis cells in a defined medium (9). The availability of this defined medium for CF production by noncompetent cells was essential for CF purification as well as for our studies on its mechanism of action. This report presents procedures for the isolation and purification of CF from culture supernatant fluids of strain Challis-6 grown in this defined medium. It also presents a partial characterization and chemical analysis of highly purified CF.

MATERIALS AND METHODS

Organisms. Strains Challis-6, SBE:12, and Wicky of group H streptococci were used. Strain Challis-6 was employed for CF production in MS6-F medium, SBE:12 resistant to 2 mg of dihydrostreptomycin/ml served as a source of deoxyribonucleic acid (DNA). Strain Wicky is a nontransformable strain which requires the addition of CF for transformation and, therefore, was used to measure CF activity (9).

Media. The compositions of the media used were as previously described (9). These media are defined medium MS6, for growth of Challis-6 cells, and defined medium MS6-F, for CF production (with the modification of tyrosine content to 2 mg/liter). In addition, Brain Heart Infusion broth (Difco) reinforced with 2.5% heat-inactivated horse serum (BHI-HS) was used for growth and transformation of strain Wicky.

Procedure for CF production in MS6-F medium. Details for CF production and all other materials and methods used, including the determination of CF biological activity by induction of Wicky cells into the competent state, were previously described (9).

CF biological activity. CF biological or transforming activity is defined as the amount of CF

"protein" (as Lowry positive material) required to transform over 2×10^6 cells of strain Wicky when 10^8 recipient cells are used in a 1-ml final volume. CF purification was based on the least amount of "protein" material required to obtain over 2×10^6 transformants per ml.

Analytical methods. Proteins were determined either by the method of Lowry (10), with bovine serum albumin as a standard, or by the spectrophotometric method (at 260 and 280 nm) of Warburg and Christian (29). Nitrogen was measured by the Johnson modification of the Nessler reaction (8). Carbohydrate tests were performed by the method of Colombo et al. (3) with the use of paper chromatography. Amino acids analyses were determined in a Hitachi-Elmer-Perkins KLA-3B amino acid analyzer through the courtesy of Jules Gladner (National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.). Phosphorus was determined as described by Chen et al. (2). Samples for lipid analysis were subjected to thin-layer chromatography on replicate silica gel plates (uniplates, Analtech Inc., Wilmington, Del.) for 100 min with chlorform-methanol-water (65:25:4) as the solvent. Lipid spots were detected by spraying the dried plates with a solution of anisaldehyde-glacial acetic acid-sulfuric acid (1:97:2) followed by heating at 100 C for 20 min (20). Replicate thin-layer plates were sprayed for phospholipids (5) and for glycolipids (28). In addition, another replicate plate was chromatographed and air-dried, and 1 cm square sections of the silica gel were tested for CF activity. Equivalent amounts of chromatographed silica gel did not affect known CF activity.

Electrophoresis techniques. Polyacrylamide gel electrophoresis was performed essentially as described by Davis (4) on 7% gels. Tris(hydroxymethyl) aminomethane (Tris)-glycine buffer (*p*H 8.6) was used with no stacking gel (4.5 ma/75 by 6 mm tube were used). The gels were stained with 0.25% Coomassie Blue; duplicate sample gels were sliced into 1-cm sections, and the gel sections were assayed directly for CF activity. Efforts to elute CF from the gel were not successful, but the use of the piece of gel directly in transformation tests gave satisfactory results for CF activity. In addition, CF homogeneity was tested by acrylamide gel electrophoresis after treatment with sodium dodecyl sulfate (SDS) as described by Maizel (11) and detailed in the text. Electrophoresis on cellulose acetate was performed by use of 2.5 by 17 cm strips (Schleicher and Schuell Co., Keene, N.H.) in a Gelman Chamber (Gelman Instruments, Ann Arbor, Mich.); sodium barbital buffer (0.05 M, *p*H 8.6) or glycine buffer (0.05 M, *p*H 10 or 10.6) was used. As a rule, 1.5 ma were used per strip. The strips were stained overnight with 0.0025% solution of nigrosin in 2% acetic acid. Again, since CF was difficult to elute from the paper, its electrophoretic mobility was determined by testing 1-cm sections of the strips directly in transformation tests. CF was tested by electrophoresis on cellulose acetate before and after SDS treatment as described above.

Electrofocusing. The principle and application of this method have been described (27). An electrofocusing column (LKB 8101) of 110 ml capacity and 1% carrier ampholite solutions were used (LKB Instruments Inc., Sweden). The ampholytes were selected to give a *p*H gradient between *p*H 3 and 10 or between *p*H 7 and 10. The CF sample was used to replace 4 ml of the less dense sucrose solution in two tubes of the middle fractions. A stepwise sucrose gradient was arranged from 0 to 50%. The cathode electrolyte solution was placed at the top of the column. Electrofocusing was performed for 40 to 60 hr with a final potential of 600 v at 6 C. Fractions of 2 ml were collected manually from the top of the column. The *p*H of each fraction was measured at 6 C. The CF biological activity was tested for directly by use of 0.2 ml (or a dilution thereof) from each tube. The ampholytes did not influence the biological activity of CF.

RESULTS

CF production and isolation from MS6-F. CF was produced by Challis-6 cells in MS6-F medium and isolated as detailed in Fig. 1. It was found that the culture supernatant fluids must be adjusted to about *p*H 6.1 prior to saturation with $(NH_4)_2SO_4$ if maximal precipitation of the small amounts of CF was to be obtained. At *p*H values below *p*H 5.0 or above *p*H 7.5, CF recovery was poor. As a rule, 5 mg (dry weight) of crude CF (3 mg of Lowry positive material) was obtained.

CF purification. Figure 2 presents the CF purification procedure developed. The procedure was divided into three steps, and the product obtained after each step was analyzed.

Step 1. The $(NH_4)_2SO_4$ precipitates from about 100 liters of culture supernatant fluids were partly solubilized at *p*H 9.0 with stirring. Most of the CF was complexed with impurities and precipitated out of solution at *p*H 3.5. By repeated precipitation at *p*H 3.5, many impurities were removed, such as $(NH_4)_2SO_4$ and

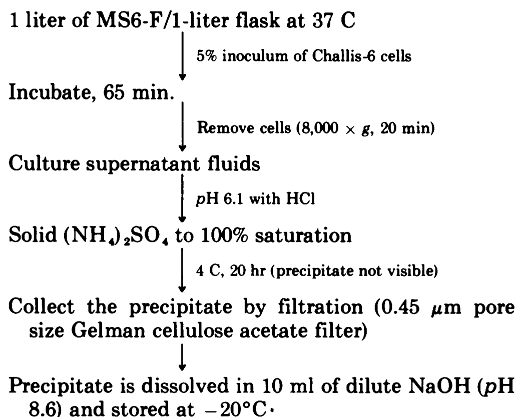

FIG. 1. *Competence factor production and isolation from MS6-F medium.*

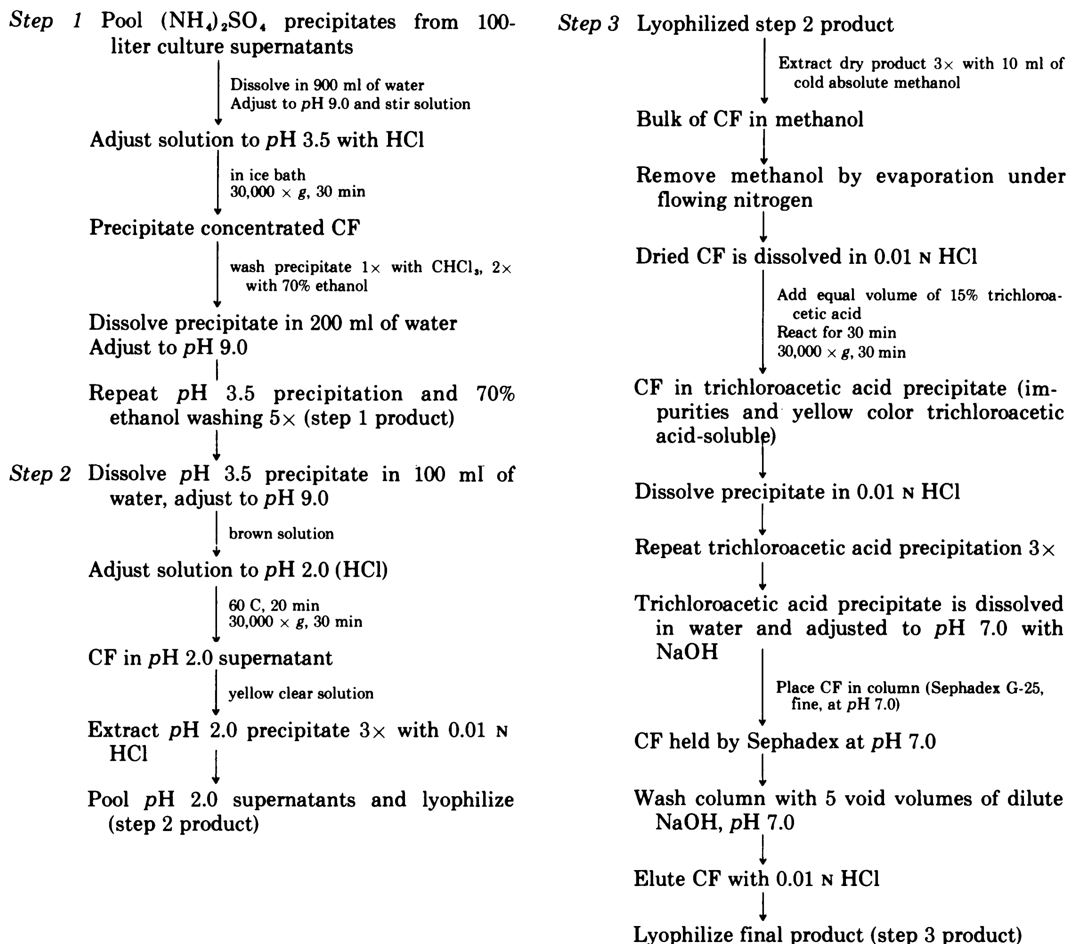

FIG. 2. *Purification of competence factor (CF).*

medium components. In addition, by washing the CF complex (*p*H 3.5 precipitate) with cold chloroform and then with 70% ethanol, materials soluble in these solvents, such as some lipids and carbohydrates were removed.

Step 2. The washed brown precipitate was dissolved at *p*H 9.0, and the solution was then adjusted to *p*H 2.0 with HCl and heated at 60 C for 20 min. This treatment solubilized most of the CF, and the bulk of the brown precipitate remained in the precipitate. The brown precipitate was re-extracted three to five times at *p*H 2.0 followed by the treatment at 60 C. The *p*H 2.0 supernatant fluids were pooled and lyophilized.

Step 3. The lyophilized CF product was then extracted three times with 10 ml of cold absolute methanol. The bulk of the CF activity was methanol-soluble, whereas significant amounts of impurities remained insoluble. The CF methanol solution was dried by vacuum distillation under flowing nitrogen until all of the methanol was removed. The dried CF, insoluble above *p*H 7.0, was dissolved in dilute HCl (*p*H 2.0), and the CF was precipitated by treatment with an equal volume of 15% trichloroacetic acid at room temperature for 30 min. The yellow-colored material containing impurities remained in the trichloroacetic acid solution. The precipitate was dissolved in dilute HCl at *p*H 2.0, and the trichloroacetic acid precipitation was repeated twice. The CF after trichloroacetic acid treatment was further purified by chromatography in a column of Sephadex G-25, 1.5 by 30 cm (fine grade Pharmacia Fine Chemicals, Inc., New York, N.Y.). The column was prewashed with 0.1 N HCl and then washed with dilute NaOH (*p*H 7.0) until the eluate was neutral. The CF solution was adjusted to *p*H 7.0 and applied to the

column. At pH 7.0, CF was bound tightly by Sephadex, and other impurities including salts, trichloroacetic acid, and denatured products were washed off the column with five void volumes of dilute NaOH, pH 7.0. The CF was then eluted from the Sephadex with 0.01 N HCl and lyophilized.

Table 1 shows the CF recovery after the various purification steps described in Fig. 2. The procedure described yielded over 1,000-fold purification of CF based on the amounts of CF "protein" (as Lowry positive material) required to obtain maximal biological activity. That is, the purest product obtained had a biological activity of 2×10^6 transformants/0.036 μg of "protein," as compared to 39 μg of "protein" in the crude material. About 2% recovery of the biological activity was obtained based upon per unit volume of the starting culture supernatant fluid.

Table 2 shows a partial characterization of CF after the three different steps in purification. The highly purified step 3 product (the purest to date) is colorless, is precipitated by 7.5% trichloroacetic acid at room temperature, and is dialyzable. It is insoluble in chloroform or ethanol but is soluble in absolute methanol. Its biological activity is stable to heating at 100 C for 10 min at pH 2.0, but is less stable at this temperature at pH 9.5. The highly purified CF remains in solution from pH 2.0 to 11.5. The biological activity of the step 1 and step 2 products was destroyed by trypsin but not by ribonuclease, deoxyribonuclease, lipase, or lysozyme. Similar effects of proteolytic enzymes on cruder CF preparations were shown by other investigators (6, 9, 25). In addition, the biological activity of the final step 3 CF product was also destroyed by trypsin. The effect of the other enzymes will be tested on the step 3 product when more material is available.

Table 3 shows a partial chemical analysis of CF after the three different steps of purification. The final purified CF (step 3 product) is composed of 90.1% "protein" (as Lowry posi-

TABLE 1. *Purification of competence factor*

Purification step	Recovery from 1 liter of culture supernatant fluids		Biological activity[a]	
	Dry wt (mg/liter)	Protein (mg/liter)	Protein (μg)	Recovery[b] (%)
Crude $(NH_4)_2SO_4$ precipitate	5.02	3.40	39.0	100
pH 3.5 precipitate (1×)	1.80	1.01	26.0	72
Step 1 product: pH 3.5 precipitate and solvent treatment (5×)	1.20	0.85	5.3	65
Step 2 product: pH 2.0 supernatant	0.40	0.36	1.0	28
Trichloroacetic acid precipitate		0.19	0.5	18
Step 3 product: pH 2.0 Sephadex eluate		0.02	0.036	2

[a] The biological activity is defined as the amount of competence factor protein (as Lowry positive material) required to render into transformability over 2×10^6 cells of strain Wicky/ml.
[b] The per cent recovery was based upon per unit volume of the starting culture supernatant fluid.

TABLE 2. *Partial characterization of competence factor*

Test performed	Step 1 product (pH 3.5 precipitate, 5×)	Step 2 product (pH 2.0 supernatant)	Step 3 product (Sephadex pH 2.0 eluate)
Color of solution	Brown	Yellow	Colorless
Trichloroacetic acid precipitation		Precipitated by 7.5% trichloroacetic acid	Precipitated by 7.5% trichloroacetic acid
Dialysis	Not dialyzable	Dialyzable	Dialyzable
Heat stability, pH 2.0	Stable	Stable	Stable
Heat stability, pH 9.5	Stable	Moderately stable	Unstable
Solubility in:			
Chloroform	Insoluble	Insoluble	Insoluble
Methanol	Insoluble	Soluble	Soluble
Ethanol	Insoluble	Insoluble	Insoluble
pH 2.0	Insoluble	Soluble	Soluble
pH 11.5	Soluble	Insoluble	Soluble

TABLE 3. *Partial chemical analysis of competence factor*

Analysis performed	Step 1 product (pH 3.5 precipitate, 5×)	Step 2 product (pH 2.0 supernatant)	Step 3 product (Sephadex pH 2.0 eluate)
Protein (Lowry positive material)	56.0%	71.1%	90.1%
Protein (OD 260 and 280 nm)		ND[a]	ND
Organic nitrogen	5.1%	9.3%	15.0%
Carbohydrates	12.2%	7.5%	ND
Phosphorus	5.0%	2.6%	ND
Lipids	1.7%	0.2%	ND
Ash	8.0%	5.8%	ND
Bound amino acids	19	12[b]	
Molecular weight			±5,000
Electrophoresis bands in:			
Acrylamide gel	6	1	1
SDS acrylamide gel			1
Cellulose acetate	4	1	1
SDS cellulose acetate			1
Electrofocusing		5	1
Electrophoretic mobility (pH 8.6)			
Acrylamide gel	None	None	None
Cellulose acetate	None	None	None
Electrofocusing		Basic	Basic
PI (electrofocusing)		>pH 11.0	>pH 11.0
Electrical charge (electrofocusing)		Positive	Positive

[a] None detected by methods employed.

[b] See text for list of the 12 amino acids detected in at least 0.002 μmole amounts per 1.00 μmole of competence factor analyzed.

tive material) containing 15% organic nitrogen. It has no absorbancy at 280 nm; therefore, it probably contains no significant amounts of aromatic amino acids. Amino acid analysis of a small amount of the step 2 product showed the following 12 amino acids (in amounts of at least 0.002 μmole per μmole of CF analyzed): aspartic acid, glutamic acid, threonine, serine, alanine, glycine, valine, lysine, arginine, histidine, leucine, and isoleucine. Tryptophan was not tested. In addition, significant amounts of ammonia were detected; thus, it is probable that compounds such as asparagine or glutamine may have been presented. When the step 2 product was later subjected to electrofocusing, at least five components were separated. Hence, we may conclude only that the factor itself contains no more than 12 amino acids in significant amounts. Further work on its amino acid composition will be performed when sufficient amounts of highly purified (step 3) CF are obtained.

Table 3 shows also that highly purified CF contains no detectable phosphorus, lipids, phospholipids, or glycolipids. In addition, when duplicate silica gel plates chromatographed in the same lipid solvent were air-dried and 1-cm square sections were tested directly for CF activity, the CF activity remained at the origin. Therefore, CF is not soluble in this lipid solvent.

Although glucose, galactose, and mannitol were found in less purified CF (step 2), preliminary chromatography tests for carbohydrates were negative when the highly purified CF (step 3) was tested by Colombo's method (3). Future carbohydrate tests will be performed by the more sensitive technique of gas chromatography.

CF (step 3) has a molecular weight of about 5,000 or less since it appears to be included in Sephadex G-25 when CF is applied to the columns at pH 2.0 (at pH 2.0, CF does not seem to bind to Sephadex). Also, the molecular weight of step 2 product based on its amino acid composition was 4,200. A similar molecular weight for CF has been reported (13).

The data obtained by electrophoresis were the most significant. The various electrophoresis techniques used are described in Materials and Methods. First, CF electrophoretic mobility could not be determined by acrylamide gel, cellulose acetate, or paper electrophoresis, since CF (including the step 3 CF) binds to the origin when tested by these electrophoresis techniques. Therefore, CF appeared neutral at all pH levels tested (pH 4.5 to 10.6). Although CF binds very tenaciously to

these substances, its location at the origin could be detected by sectioning into 3-mm sections the gels or the paper or cellulose acetate strips and adding the sections directly to transformation test containing Wicky cells and DNA. In all tests performed, all of the biological activity was found at the origin. By electrofocusing, however, it was possible to determine both the purity and the isoelectric point of CF. Table 4 shows a typical electrofocusing run; a 1% solution of ampholine (*p*H 7 to 10) with an initial average *p*H value of *p*H 8.6 was used (see Materials and Methods for details). With the step 2 product (*p*H 2.0 supernatant fluid; Table 4), precipitates appeared at the onset of the experiment since such CF preparations precipitate above *p*H 7.0 All of these precipitates and yellow color moved toward the anode at *p*H 8.6. Some CF activity was bound or occluded with these precipitates, and low levels of CF activity were found in most fractions. At least five different visible bands were obtained. These five visible bands or precipitates gave no absorbancy at 260 to 280 nm. The same preparations had shown only one protein band by polyacrylamide gel or cellulose acetate electrophoresis (Table 3). As shown in Table 4, the bulk of CF activity moved into the *p*H 11.0 range (the cathode solution) but was not detectable visually by either color or precipitation. As shown in Table 4, the step 3 CF gave no visible bands or precipitate in the column. Also, none of the fractions from the column gave absorbancy at 260 to 280 nm. Since all of the CF activity moved into the cathode solution above *p*H 11.0 and out of the *p*H range of the ampholines, its exact isoelectric point, which is above 10.0, could not be determined. But it can be concluded that CF is positively charged, is extremely basic, and has an isolelectric point above *p*H 10.0 and probably above *p*H 11.0.

The step 3 CF appeared to be homogeneous by electrofocusing, since no other substances were detected in the column. We tested the step 3 CF for homogeneity after treatment with 1% SDS at 37 C for 2 hr or at 100 C for 2 min according to Maizel's method (11), using both acrylamide gel and cellulose acetate electrophoresis. In both cases, only one protein band was detected (Table 3). This protein band contained all of the CF activity. As expected, the CF after treatment with the anionic SDS became negatively charged (30).

TABLE 4. *Electrofocusing of competence factor*[a]

Tube no.	*p*H	Step 2 product[b]		Step 3 product[b]	
		Visible bands	CF activity[c]	Visible bands	CF activity[c]
1	12.41	None	–	None	–
2	12.41	None	–	None	–
3	12.40	None	2+	None	+
4	11.97	None	4+	None	2+
5	11.85	None	4+	None	4+
6	11.67	None	4+	None	4+
7	11.20	None	2+	None	4+
8	11.00	None	2+	None	2+
9	10.83	None	+	None	+
10	9.75	None	+	None	+
11	9.59	None	+	None	–
12	9.32	None	+	None	–
13	9.27	Yellow band	+	None	–
14	9.20	Yellow band	+	None	–
17	9.15	Yellow band	+	None	–
19	9.02	Yellow band	+	None	–
20	9.01	Precipitate	+	None	–
22	8.84	Precipitate	+	None	–
24	8.60	None	+	None	–
26	8.40	Precipitate	+	None	–
28	8.12	Precipitate	+	None	–
30	7.70	Precipitate	+	None	–
32	7.12	None	–	None	–
34	6.50	Brown band	–	None	–
36	6.33	Yellow band	–	None	–
40	1.60	None	–	None	–
50	1.60	None	–	None	–

[a] LKB ampholine, *p*H 7 to 10 (1% solution), was used with the cathode as the upper electrode. Column was emptied from the top, and 2-ml samples were collected per tube.

[b] A 4-ml amount of either step 2 product (after *p*H 2.0 heat extraction) or step 3 product (Sephadex *p*H 2.0 eluate) was used. The CF was placed in the middle of the column during the manual preparation of the sucrose gradient.

[c] Competence factor activity was measured from + to 4+ with increasing activity and negative (–) if no activity was detected with up to 0.2-ml samples. The ampholytes did not influence the biological activity of competence factor.

DISCUSSION

The extremely small amounts of CF produced by Challis cells, its apparent instability, and the strong tendency of CF to absorb to some proteins, glass, cellulose, Sephadex, acrylamide gel, and other chromatographic materials have long hampered its purification and identification.

This study was directed to the development of conditions for the isolation and purification of CF produced by Challis-6 cells in defined medium. The procedure described here yields CF of very high purity, if not pure. This report also presents the first extensive characterization and chemical analysis of the highly purified CF.

The most significant finding was its extremely high isoelectric point of above *p*H 11.0. The fact that the purified CF has such a high isoelectric point, that it is sensitive to trypsin, that it is precipitable by trichloroacetic acid, and that it lacks detectable amounts of sulfur-containing amino acids suggests the possibility that CF maybe a protamine or protamine-like compound rich in arginine or lysine, or both. CF is unlikely to be a free polyamine, since it is precipitated by trichloroacetic acid and is sensitive to trypsin. However, a polyamine could be a part of a larger macromolecule, with a peptide acting as a carrier for the polyamine.

Since the chemical nature of CF remains uncertain, we based its purification on the least amounts of CF "protein" (as Lowry positive material) required to obtain maximal biological activity. Since other compounds beside proteins react with the Folin phenol reagent (10) and, moreover, purified CF showed no absorbancy at 280 nm, we define CF "protein" in terms of Lowry positive material. Therefore, until sufficient amounts of highly purified CF are analyzed for amino acids, and an organic nitrogen, amino nitrogen, and dry weight balance are obtained, the final characterization of CF cannot be made. Studies along these lines are in progress.

Once CF is finally characterized, studies on its mechanism of action can be approached in a more comprehensive and meaningful manner. Nonetheless, the fact that CF is such an extremely basic compound suggests several possible roles, such as altering the bacterial surface charge, as well as affecting other processes required to bind the highly acidic DNA irreversibly at the membrane level.

Evidence for an initial effect on the cell surface charge after the binding of exogenous CF to cells exists. Pakula et al. (17) showed a decrease in the ability of cells to adsorb methylene blue after CF adsorption and competence development. Similar results were obtained with competent *Bacillus subtilis* cells by Jensen and Haas (7). This modification of the negatively charged cell wall should make the cell more receptive to the reversible binding of exogenous DNA. The location of the receptor site for the initial and reversible binding of CF is not known. It may be at the cell wall level or possibly at the membrane level, as described by Ziegler and Tomasz for pneumococci (31). The initial CF-cell binding does not require protein synthesis in either pneumococci (31) or streptococci (Leonard, *unpublished data*). But it is known that exogenous CF, after its binding to the cell, induces synthesis of new proteins, and that these proteins are required for competence development (9, 15, 22). The nature and role of these proteins are not known. It is possible that one of the induced proteins is the autolysin described by Ranhand et al. (19). Another protein induced by CF is a structural competence-specific protein described by Pakula et al. for streptococci (17) and by Tomasz and Zanati for pneumococci (26). It is possible that this structural protein or some other protein induced by CF may be the irreversibly binding receptor site for CF itself at the membrane level. This cell membrane-CF complex may in turn function as the protein receptor site for irreversible binding of DNA at the membrane. Such a DNA-binding protein has been described by Alberts and Frey in T4 bacteriophage-infected cells of *Escherichia coli* (1). These investigators showed that this DNA-binding protein is also involved in DNA replication and genetic recombination. That membrane-bound CF may be the DNA receptor site has been suggested by other investigators from data obtained with antiserum to competent and noncompetent cells in both streptococci (14, 27) and pneumonococci (12, 23, 25). The concept that CF may be an integral part of the irreversible binding site for DNA on the membrane is further reinforced by our present finding that CF is such an extremely basic compound. Studies on the identification of such a DNA receptor site are in progress.

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LITERATURE CITED

1. Alberts, B. M., and L. Frey. 1970. T_4 bacteriophage 32: a structural protein in the replication and recombination of DNA. Nature (London) **227**:1313–1318.
2. Chen, P., T. Toribara, and H. Wagner. 1956. Microdetermination of phosphorus. Anal. Chem. **28**:1756–1758.
3. Colombo, P., D. Corbetta, A., Pirotta, G. Ruffini, and A. Sartori. 1960. A solvent for qualitative and quantitative determination of sugars using paper chromatography. J. Chromatogr. **3**:343–350.
4. Davis, B. J. 1964. Dish electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**:404–427.
5. Dittmer, J. C., and R. L. Lecter. 1964. A simple, specific spray for the detection of phospholipids on thin layer chromatograms. J. Lipid Res. **5**:126–127.
6. Dobrzanski, W. T., and H. Osowiecki. 1967. Isolation

and some properties of the competence factor from Group H *Streptococcus* strain Challis. J. Gen. Microbiol. **48**:299-304.

7. Jensen, R. A., and F. L. Haas. 1963. Electrokinetics and cell physiology. I. Experimental basis for electrokinetic cell studies. J. Bacteriol. **86**:73-78.
8. Johnson, M. J. 1941. Isolation and properties of a pure yeast polypeptidase. J. Biol. Chem. **137**:575-586.
9. Leonard, C. G., J. M. Ranhand, and R. M. Cole. 1970. Competence factor production in chemically defined media by noncompetent cells of group H *Streptococcus* strain Challis. J. Bacteriol. **104**:674-683.
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
11. Maizel, J. V. 1969. Acrylamide gel electrophoresis of proteins and nucleic acids, p. 334-362. *In* K. Habel and N. R. Salzman (ed.), Fundamental technique in virology. Academic Press Inc., New York.
12. Nava, G., A. Gallis, and S. Beiser. 1963. Bacterial transformation: an antigen specific for competent pneumococci. Nature (London) **197**:903-904.
13. Osoweicki, H., J. Nalecz, and W. T. Dobrzanski. 1969. The mechanism of competence in the transformation of Streptococci of serological Group H. Mol. Gen. Genet. **105**:16-20.
14. Pakula, R. 1965. Inhibition of transformation of streptococci by antibodies. J. Bacteriol. **90**:1501-1502.
15. Pakula, R., J. Cybulska, and W. Walczak. 1963. The effect of environmental factors on transformability of a streptococcus. Acta Microbiol. Pol. **12**:245-248.
16. Pakula, R., M. Piechowska, E. Bankowska, and W. Walczak. 1962. A characteristic of DNA mediated transformation systems of two streptococcal strains. Acta Microbiol. Pol. **11**:205-222.
17. Pakula, R., P. Ray, and L. R. Spencer. 1970. Some characteristics of streptococci competent for uptake of deoxyribonucleic acid. Can. J. Microbiol. **16**:345-350.
18. Pakula, R., and W. Walczak. 1963. On the nature of competence of transformable streptococci. J. Gen. Microbiol. **31**:125-133.
19. Ranhand, J. M., C. G. Leonard, and R. M. Cole. 1971. Autolytic activity associated with competent group H streptococci. J. Bacteriol. **106**:257-268.
20. Stahl, E. 1965. Thin layer chromatography. A laboratory handbook, p. 485. Academic Press Inc., New York.
21. Tomasz, A. 1969. Some aspects of the competent state in genetic transformation. Annu. Rev. Genet. **3**:217-232.
22. Tomasz, A. 1970. Cellular metabolism of genetic transformation of pneumococci: requirement for protein synthesis during induction of competence. J. Bacteriol. **101**:860-871.
23. Tomasz, A., and S. M. Beiser. 1965. Relationship between the competence antigen and the competence-activator substance in pneumococci. J. Bacteriol. **90**:1226-1232.
24. Tomasz, A., and R. Hotchkiss. 1964. Regulation of the transformability of pneumococcal cultures by macromolecular cell products. Proc. Nat. Acad. Sci. U.S.A. **51**:480-487.
25. Tomasz, A., and J. Mosser. 1966. On the nature of the pneumococcal activator substance. Proc. Nat. Acad. Sci. U.S.A. **55**:58-64.
26. Tomasz, A., and E. Zanati. 1971. Appearance of a protein "agglutinin" on the spheroplast membrane of pneumococci during induction of competence. J. Bacteriol. **105**:1213-1215.
27. Vesterberg, O., and H. Sversson. 1966. Isoelectric fractionation, analysis and characterization of ampholytes in natural pH gradients. Acta Chem. Scand. **20**:820-834.
28. Vorbeck, M. L., and G. V. Marinetti. 1965. Separation of glycosyl diglycerides from phosphatides using silicic acid column chromatography. J. Lipid Res. **6**:3-6.
29. Warburg, O., and W. Christian. 1941. Isolierung und Kristallisation des Garungsferments Enolase. Biochem. Z. **310**:384-421.
30. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244**:4406-4412.
31. Ziegler, R., and A. Tomasz. 1970. Binding of the competence factor to receptors in the spheroplast membrane of pneumococci. Biochem. Biophys. Res. Commun. **41**:1342-1349.