Isolation of Group H Streptococcal Competence Factor

DENNIS PERRY

Department of Microbiology, Northwestern University Medical School, Chicago, Illinois 60611

Received for publication 20 June 1973

Competence factor (CF) was purified 600-fold from culture filtrates of competent group H streptococcus, strain Challis, by fractionation on a carboxymethyl-Sephadex column. The preparation did not contain orcinol- or diphenylamine-positive material, and CF activity was destroyed by trypsin. An examination of its chemical composition revealed 10 amino acids, muramic acid, glucosamine, and glucose.

The development of competence by transformable streptococci is accompanied by the production of extracellular competence factor (CF) which can induce competence in physiologically noncompetent cells of the same or closely related streptococcal strains (10). This observation subsequently led to a number of studies on the chemical nature and mechanism of action of CF. Because most investigators used culture filtrates as the source of CF, some of the results obtained in these studies might have been influenced by factors unrelated to competence induction. For example, Pakula et al. (9) and Nalecz et al. (6) have shown that culture filtrates from competent group H streptococci contain deoxyribonuclease activity. In addition, Schlegel and Slade (12) showed that such culture filtrates also contain a bacteriocin which is bacteriocidal for a noncompetent group H streptococcus. Thus, it appears obvious that purified or partly purified CF would be particularly advantageous in these studies. Osowiecki et al. (7) and Leonard and Cole (4) recently described methods for the purification of CF from culture filtrates of competent group H streptococcus, strain Challis. The latter investigators were able to obtain a CF preparation which had been purified over 1,000-fold. However, the method employed by these investigators appears less suitable for the routine isolation of CF, for it requires 100 liters of culture filtrate and involves multiple precipitation and extraction procedures prior to fractionation on an ion-exchange resin (Sephadex G25). This report describes a rapid, simple procedure for the isolation of CF from culture filtrates of competent strain Challis.

Group H streptococcus, strain Challis, was grown to competence in one liter of PYG medium as previously described (11). The bacteria were removed by filtration through type HA membrane filters (0.45 µm; Millipore Corp.) and the crude CF filtrate was precipitated at 4°C by adding (NH₄)₂SO₄, to 60% saturation (8). The resulting precipitate was collected by centrifugation, suspended in approximately 50 ml of distilled water, and dialyzed against distilled water for 18 h at 4°C. The dialyzed material was then lyophilized and stored under vacuum at 4°C. The CF activity of this crude preparation remained stable for approximately 8 weeks.

A 200-µg sample of crude, lyophilized CF was dissolved in 3 to 5 ml of 0.05 M sodium phosphate buffer, pH 8.0, and applied to a column (1.5 by 25 cm) of carboxymethyl (CM)-Sephadex (C25; Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) which had been equilibrated at 4°C with the above buffer. The column was eluted with a linear gradient between 0.05 and 0.2 M sodium phosphate buffer, pH 8.0, with a flow rate of 18 ml/h. The fractions (10 ml) were collected, and the absorbance (280 nm) and CF activity of each were determined (Fig. 1). Noncompetent group H streptococcus, strain Wicky, was used as the indicator strain for CF activity (10). Virtually all of the 280-nm absorbing material was eluted in the first 5 to 7 fractions, whereas the bulk of CF activity (transformants/milliliter) was eluted in a very sharp peak at approximately 300 ml. Fractions 27 to 31 were pooled and stored at −70°C with no appreciable loss of CF activity for 1 to 2 weeks. The CF activity of these preparations was maintained for approximately 4 weeks when 2-mercaptoethanol was added to 5 mM.

The percent recovery of several preparations of CF after elution from CM-Sephadex was 10 to 15%. In these experiments, percent recovery
was based upon the amount of CF activity in the original culture filtrate, which resulted in the transformation of 1% of a population of 10⁷ to 3 x 10⁷ Wicky colony-forming units. The amount of crude CF necessary for maximum transformation (1%) contained 366 μg of protein (5), whereas a similar amount of CF eluted from CM-Sephadex contained only 0.6 μg of protein. Therefore, the fractionation of CF on CM-Sephadex resulted in an approximate 600-fold purification based on the amount of protein associated with CF activity. These CF preparations did not contain orcinol- (1) or diethyleneamine-positive (3) material and were completely free of deoxyribonuclease and bacteriocin activity. CF activity was destroyed by trypsin. The binding of CF by CM-Sephadex at pH 8.0 (but not by diethyleneaminomethyl-Sephadex) is consistent with the findings of Leonard and Cole (4) that CF is positively charged and has a high isoelectric point.

Results of amino acid (13) and gas chromatographic (2) analyses of the composition of CF revealed the presence of 10 amino acids, ammonia, muramic acid, glucosamine, and glucose (Table 1). Leonard and Cole (4) also found arginine and histidine in their purified CF preparations in addition to the 10 amino acids found here. Carbohydrate were not detected by these investigators. It should be mentioned, however, that Leonard and Cole (4) employed conventional chemical methods to examine their CF preparation for carbohydrates as compared with the more sensitive technique of gas chromatography used in this investigation. On the other hand, the differences in CF composition may be due to the greater degree of purification obtained by these investigators.

Although the CF isolated in these studies has been purified some 600-fold, based on protein analysis, the extent of purification awaits our further investigation by using electrophoresis and electrofocusing techniques. Nevertheless, the procedure described in this investigation should be useful, not only as a preliminary step toward the isolation of CF of higher purity, but also for the routine isolation of CF for studies on its mechanism of action.

The author expresses his appreciation to Shelia N. Prachand for her valuable technical assistance.

This investigation was supported by Public Health Service grant AI-07694 from the National Institute of Allergy and Infectious Diseases.

| Component         | μmol (x 10⁻⁹/ml) |
|-------------------|------------------|
| Ammonia           | 19.6             |
| Lysine            | 4.6              |
| Aspartic acid     | 5.9              |
| Threonine         | 9.2              |
| Serine            | 17.0             |
| Glutamic acid     | 9.6              |
| Glycine           | 8.1              |
| Alanine           | 3.1              |
| Valine            | 0.4              |
| Isoleucine        | 0.1              |
| Leucine           | 0.2              |
| Muramic acid      | 3.1              |
| Glucosamine       | 18.7             |
| Glucose           | 1.5              |

LITERATURE CITED

1. Ashwell, G. 1957. Colorimetric analysis of sugars, p. 87-90. In S. P. Coldick and N. O. Kaplan (ed.), Methods in enzymology, vol. 3. Academic Press Inc., New York.
2. Bolton, C. H., J. R. Clamp, and H. L. Hough. 1965. The use of gas liquid chromatography in investigations on glycoproteins. Biochem. J. 96:5C-6C.
3. Dische, Z. 1955. Color reactions of nucleic acid components, p. 285-305. In E. Chargaff and J. N. Davidson (ed.), The nucleic acids: chemistry and biology, vol. 1. Academic Press Inc., New York.
4. Leonard, C. G., and R. M. Cole. 1972. Purification and properties of streptococcal competence factor isolated from chemically defined medium. J. Bacteriol. 110:273-280.
5. 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.
6. Nalecz, J. H., Osowiecki, and W. T. Dobrzenski. 1970. A factor inactivating in vitro transforming DNA isolated from transformable group H streptococci and its relation to competence of the cells. Bull. Acad. Pol. Sci. Ser. Sci. Biol. 18:867-869.
7. Osowiecki, H., J. Nalecz, and W. T. Dobrzenski. 1969. The mechanism of competence in the transformation of streptococci of serological group H. Purification and some properties of the competence factor. Mol. Gen.
8. Pakula, R. 1967. Cellular sites for the competence-provoking factor of streptococci. J. Bacteriol. 94:75-79.
9. Pakula, R., L. R. Spencer, and P. A. Goldstein. 1972. Deoxyribonuclease and competence factor activities of transformable and nontransformable group H streptococci. Can. J. Microbiol. 18:111-119.
10. Pakula, R., and W. Walczak. 1963. On the nature of competence of transformable streptococci. J. Gen. Microbiol. 31:125-133.
11. Perry, D. 1968. Transformability of streptomycin-resistant group H streptococci. J. Bacteriol. 95:132-138.
12. Schlegel, R., and H. D. Slade. 1972. Bacteriocin production by transformable group H streptococci. J. Bacteriol. 112:824-829.
13. Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30:1190-1206.