On the Nature of Competence of Transformable Streptococci

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SUMMARY

Transformable streptococci produce an exocellular factor provoking competence under certain conditions. Non-competent cultures become competent upon addition of this factor. The kinetics of conversion is concomitant with an enzymic reaction; the process is time and temperature dependent and the factor itself is heat sensitive. The action of the hypothetical enzyme on cells of a non-transformable streptococcus results in provocation of competence.

INTRODUCTION

The term competence is defined here as the ability to undergo transformation. Some workers define this state in terms of capacity of cells to take up DNA irreversibly and to undergo subsequently transformation. There is, indeed, no real difference between the definitions since DNA uptake is a necessary condition of genetic transformation. The definition of biochemical conditions favouring competence is of considerable interest. Transformability of bacteria is limited to some strains of a small number of species. Without an understanding of the nature of competence, extension of the phenomenon of transformation to more bacterial species can only be a matter of chance. The physiological state of competence seems to be in some way connected with the mechanism of DNA penetration through the cell wall. Two hypotheses have been proposed to account for penetration: one termed the 'localized protoplast' hypothesis and the other defined as the 'enzymic receptor' hypothesis (Ravin, 1961). The 'localized protoplast' hypothesis, based mainly on evidence provided by Thomas (1955), regards the competent cell as one having during some period of its life cycle naked areas on the surface through which DNA can penetrate. According to the second hypothesis, competent cells produce on their surface an enzyme which catalyses DNA uptake (Fox & Hotchkiss, 1957).

Pakula, Piechowska, Bankowska & Walczak (1962) reported that sterile supernatant fluids from cultures of competent streptococci contain a factor which provokes competence of homologous and related cells. Further work, presented in this paper, has shown that upon addition of this factor, transformation can occur in a medium containing neither serum nor albumin, i.e. in conditions in which transformation of streptococci never occurs or is extremely infrequent. Moreover, the factor produced by the strain Challis, a group H Streptococcus, provokes transformation of the non-transformable strain Wicky of the same serological group. The conversion of non-competent cells into competent ones seems to be concomitant with an enzymic reaction.
METHODS

Organisms. The following streptomycin-sensitive strains of group H Streptococcus were used as recipients: Challis, 3437/48, Wicky. The action of the provoking factor was also tested on some other strains of group H Streptococcus as well as on strains of Streptococcus sanguis, type I, type II and type I/II. All the group H streptococci were obtained from the Streptococcal Reference Laboratory, Colindale, London. The strains of S. sanguis were given us by Dr R. Wahl of the Pasteur Institute in Paris.

The strains Challis and Streptococcus sanguis, type I/II, can be transformed to streptomycin-resistance with rather high efficiencies and have been used in this laboratory in transformation studies (Pakula, Hulanicka & Walczak, 1958). Strain 3437/48 was recently found to be transformable.

Media. Two media were used for the production of transformants and of the factor which provokes transformation. One medium (ST) contained beef heart extract, prepared as for Todd-Hewitt broth (1932), Difco neopeptone 1%, charcoal-adsorbed Difco yeast extract 0.5%, glucose 0.2%. The second medium (ET 3) contained distilled water, Difco neopeptone 1%, charcoal-adsorbed Difco yeast extract 0.5%, sodium chloride 0.85%, glucose 0.2%. Both media were at pH 7.4-7.5.

Transforming DNA. One DNA preparation, derived from a mutant of strain Challis resistant to 2 mg. streptomycin/ml., was used throughout this work. The efficiencies of the interspecific transformations to streptomycin-resistance of the heterologous streptococcal strains with this DNA are of the same order of magnitude as those obtained with the corresponding homologous DNA preparations. The procedure of DNA extraction and purification was reported elsewhere (Pakula et al. 1962).

Competent cells. Under normal conditions, i.e. without addition of the provoking factor, competent cells of the three transformable strains: Challis, 3437/48 and Streptococcus sanguis, type I/II, cannot be produced in either of the above two media, unless swine serum, previously heated for 30 min. at 62° to destroy DNAase activity, or albumin is added, this is absolutely necessary to produce any significant transformation. However, upon addition of active culture filtrates or of crude concentrates thereof, transformation can be obtained, in some instances, in medium ET 3 containing neither serum nor albumin. In order to obtain non-competent cells for the provocation test, 50 ml. of this medium was inoculated with 2.5 ml. of an 18 hr. blood broth culture previously diluted 1/250. With this size of inoculum initial concentrations of 5 to 7 x 10^4 cocci/ml. were found. After 3 hr. of growth at 37° in aerobic conditions the non-competent culture was converted to competence by addition of the provoking factor as described below.

Preparation of active supernatant fluids and of crude concentrates of the provoking factor. Bacteria grown in any medium which permits transformation of a given strain produce the factor which enhances or provokes competence. The presence of this factor seems to be a minimum requirement for competence of a streptococcal culture. Cultures were centrifuged and the supernatant fluids were filtered through a G5 glass filter and tested for sterility.
Crude concentrates of factor were obtained by precipitation with ammonium sulphate or ethanol. A cellophan tube was filled with a given volume of the sterile supernatant fluid and placed in a glass vessel containing 10 volumes of a saturated solution of ammonium sulphate. After 18–24 hr. at 4° a precipitate was formed within the tube. Ethanol precipitates were formed after addition of 1 volume of supernatant fluid to 8 volumes of cold ethanol (−10°). Both kinds of precipitates were dissolved in 0.01 M-phosphate buffer (pH 7).

Activity tests of provoking factor. Induction of competence was demonstrated under two different conditions: (1) in medium ET3 not containing either serum or albumin; (2) in medium ST+serum. In the first case, competence appeared only on adding active supernatant fluid, or concentrate. As concerns the second case, it was shown by Pakula et al. (1962) that the time of appearance of competence of streptococci, in media containing serum or albumin, was dependent on the inoculum size. The smaller the inoculum the later the appearance of competence. The activity of the provoking factor can, therefore, be shown in cultures started with a small initial inoculum when the factor is added before natural competence occurs.

Activity of the provoking factor was measured by exposing to it for 30 min. a 3 hr. non-competent culture of an initial density mentioned above, 5 µg. DNA/ml. was then added and after 10 min. any non-incorporated DNA was destroyed by adding DNAase. Bacteria were grown for the next 2 hr. to allow phenotypic expression of the acquired streptomycin-resistance character. Transformants were scored on blood agar plates containing 250 µg. streptomycin/ml.

RESULTS

Development of competence and production of the provoking factor

Figure 1 illustrates the time-dependence of competence and the production of provoking factor by a culture of strain Challis in ST medium + 5% swine serum. With an initial concentration of 0·5 to 1 × 10⁶ cocci/ml., maximum competence was achieved after about 3 hr. The frequency of transformants at this time was 8–22% of the total number of cocci. After incubation for 7–8 hr. competence disappeared completely.

At the times indicated in Fig. 1, samples of culture were collected, sterile supernatant fluids prepared from them and their ability to provoke competence tested on bacteria cultivated in medium ET3 without serum or albumin. As mentioned above, under these conditions no transformants are produced in absence of provoking factor. It can be seen in Fig. 1 that the activity of culture supernatant fluids increased initially with the increase of competence. However, while competence disappeared at the end of the logarithmic phase of growth, the activity of culture supernatant fluids prepared after incubation for 17 hr. did not differ markedly from those prepared at the time of maximum competency.

The addition of 5–10% (v/v) of an active supernatant fluid to bacteria incubated in medium ET3 for 3 hr. followed by incubation of the mixture for 30 min., resulted in transformation of 5–15% of the entire population.

Medium ST and medium ET3, supplemented with serum or albumin, are both suitable for transformation of organisms of strain Challis and for the production of provoking factor. Transformation of Streptococcus sanguis type I/II was also
observed in these media but only in the presence of serum, not of albumin. Medium ET3, supplemented with serum, permitted transformation of strain 3437/48. The efficiencies of transformation of *S. sanguis* type I/II and of strain 3437/48, as a percentage of entire population, were 3–8% and 2–4%, respectively.

*The activity of supernatant fluids and of crude concentrates of provoking factor*

Figure 2 illustrates the provocation of competence of organisms of strain Challis, grown in medium ET3 without serum or albumin, when homologous supernatant fluid and crude concentrates were added. The concentrates were obtained by precipitation with ammonium sulphate, as mentioned above, or with 8 volumes of ethanol, an amount found to be optimal. It may be seen that the effect was dependent on the amount of provoking factor added. Curves C and E, illustrating

![Figure 1](image1.png)

**Fig. 1**

Development of competence of *Streptococcus* strain Challis, in medium ST+ serum, and competence-provoking activities of supernatant fluids prepared at times indicated. The competence-provoking activity of supernatant fluids was tested on homologous organisms grown in medium ET3. ●—●, competence; ×—×, competence-provoking activity of the supernatant fluid.

![Figure 2](image2.png)

**Fig. 2**

Competence-provoking activities of a supernatant fluid and of crude concentrates of provoking factor. A, supernatant fluid; C, ammonium sulphate precipitate dissolved in 1/10 of the initial volume; E, ethanol precipitate dissolved in 1/10 of the initial volume; B, ammonium sulphate precipitate dissolved and diluted to the initial volume of the supernatant fluid used for precipitation; D, ethanol precipitate dissolved and diluted to the initial volume of the supernatant fluid used for precipitation.

the development of competence following the addition of the concentrates, follow the same line. The smallest amounts of the concentrates used were found to be saturating. From these curves it might be concluded that the efficiencies of both methods of concentration are equal. However, this is not true, as can be seen from curves B and D, which show the activities of concentrates diluted to the initial volume of the supernatant fluid used for concentration. It is evident that precipitation with ammonium sulphate was much more effective than precipitation with ethanol.
Heat sensitivity of the provoking factor

Concentrates of the factor which provokes competence were diluted either in medium ET3 or in 0.01 M-phosphate buffer (pH 7) or in saline. Samples of all these solutions were heated for 10 min. at various temperatures (see Fig. 3) and their ability to provoke competence tested on strain Challis organisms grown in medium ET3 without serum or albumin. Competence in this condition was solely due to addition of provoking factor since transformation was never observed in controls not supplemented with this factor. Obviously, non-saturating amounts of provoking factor were added in order to observe any decline of activity caused by heating. Samples of provoking factor diluted before heating in medium ET3 were more resistant than those diluted in buffer or saline. The provoking factor seems to be protected by some organic compounds present in the medium.

Fig. 3. Heat sensitivity of competence-provoking factor. The activity of the unheated diluted concentrate is indicated as 100%. -x-x, Concentrate diluted in medium ET3 before heating; o-o, concentrate diluted in buffer before heating; △-△, concentrate diluted in saline before heating. All samples were heated 10 min. at the temperatures shown.

Fig. 4. Dependence of activity of the competence-provoking factor on temperature.

Temperature dependence of the activity of the provoking factor

A 3 hr. culture of strain Challis growing in medium ET3 was divided into several samples, each adjusted to one of the temperatures indicated in Fig. 4 and supplemented with the factor which provokes competence. This factor was allowed to act on organisms at the indicated temperatures for 30 min. At the end of this period, all the samples were transferred to a 37° water bath, exposed to DNA for 10 min. and incubated for 2 hr. to permit phenotypic expression of transformants. As shown in Fig. 4, the provoking factor was not active at 0° and 10°; maximum activity was at 37°. Temperatures higher than 40° could not be used because they affected growth and viability.

G. Microb. xxxi
Time of action of provoking factor and efficiency of transformation

A 3 hr. culture of strain Challis growing in medium ET3 was divided into equal samples. Equal amounts of provoking factor were then added to each sample at various times, so that the bacteria in each sample had been grown for the same time but the cocci were exposed to the provoking factor for different times. After application of the normal procedure, the number of transformants in each sample was determined. As indicated in Fig. 5, the optimal period of action of provoking factor was 25–35 min. The mean generation time of strain Challis organisms in medium ET3 is 33 min.

Fig. 5. Dependence of activity of the competence-provoking factor on the time of contact with the organisms before DNA addition.

Fig. 6. Effect of dialysis on the activity of competence-provoking factor. ×—×, activity of the dialysed concentrate diluted in buffer; ▲—▲, activity of the dialysed concentrate diluted in buffer used for dialysis; ●—●, activity of the non-dialysed concentrate diluted in buffer.

Dialysis of the factor which provokes competence

Visking dialysis tubing, declared to hold back particles of molecular weight greater than $10^4$, were filled with a given volume of a concentrate of provoking factor and placed in a glass vessel containing 20 volumes of 0.01M-phosphate buffer (pH 7). After dialysis at $4\degree$ for 48 hr., samples of the dialysis residue were appropriately diluted in fresh buffer and in the buffer which had been used for the dialysis. A non-dialysed sample of the batch of provoking factor concentrate was diluted in the same way. The activity of these samples was tested on organisms.
grown in medium ET3. The results, given in Fig. 6, show a slight loss of activity after dialysis. This loss was not restored by dilution of the dialysis residue in the buffer used for dialysis which suggests that no co-factor was necessary for the action of the hypothetical enzyme. This kind of experiment was repeated several times. The loss of activity varied from experiment to experiment but was never very significant. Some variation might have been due to the inaccuracy of the test used for measuring of activity of provoking factor.

The specificity of action of provoking factor

Samples of competence-provoking factor were prepared from cultures of the three transformable strains, Challis, Streptococcus sanguis type I/II and 3437/48. Concentrates of these preparations were diluted appropriately and their capacities to provoke transformation tested on these transformable strains as well as on the following non-transformable strains of group H Streptococcus and S. sanguis: Wicky, E91/46, Channon, Blackborn, H4, S. sanguis, types I and II. Provocation of competence was observed in medium ET3 and in medium ST+swine serum. In the latter medium, organisms grown only for 2–2½ hr. were used for test, that is, before competence naturally occurred or, if it did, was of very low degree.

Table 1. Specificity of action of competence-provoking factors derived from cultures of transformable strains of Streptococcus: Challis; S. sanguis type I/II; 3437/48

Test organisms grown in medium ET3 without serum or albumin.

<table>
<thead>
<tr>
<th>Provocation of competence tested on Streptococcus strains</th>
<th>Control without provoking factor</th>
<th>Activity of provoking factors derived from cultures of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S. sanguis type I/II 3437/48</td>
</tr>
<tr>
<td>Challis</td>
<td>0</td>
<td>1 x 10^4 0</td>
</tr>
<tr>
<td>S. sanguis type I/II</td>
<td>0</td>
<td>2 x 10^4 0</td>
</tr>
<tr>
<td>3437/48</td>
<td>0</td>
<td>21 x 10^4 0</td>
</tr>
<tr>
<td>Wicky</td>
<td>0</td>
<td>52 x 10^4 0</td>
</tr>
<tr>
<td>E 91/46</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>Channon</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>Blackborn</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>H4</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>S. sanguis type I</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>S. sanguis type II</td>
<td>0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

Provocation of competence in medium ET3 is illustrated in Table 1. It can be seen that the factor derived from a culture of strain Challis provoked competence of homologous organisms and of organisms of strains 3437/48 and Wicky. The latter strain could not be transformed in the normal way under various conditions found successful with other streptococci. The action of the factor from Challis cultures on Streptococcus sanguis type I/II was negligible. The factor from cultures of strain 3437/48 was not active at all under these conditions.

Table 2 illustrates the enhancement of competence in medium ST+serum. In this case, the factor derived from a culture of Streptococcus sanguis type I/II
enhanced competence not only of homologous organisms but also those of strain Challis, 3437/48 and Wicky. The factor from cultures of strain 3437/48 acted on homologous organisms and on organisms of the group H streptococci Challis and Wicky, but not on S. sanguis type I/II. The same batch of provoking factor was used in the experiments recorded in Tables 1 and 2. It may be pointed out that we succeeded in provoking competence of the probably non-transformable strain Wicky, but we were not able to provoke competence of the other strains of group H streptococci and of S. sanguis listed in the Tables.

Table 2. Specificity of action of competence-provoking factors derived from cultures of the transformable strains of Streptococcus: Challis; S. sanguis type I/II; and 3437/48

<table>
<thead>
<tr>
<th>Test organisms grown in medium ST with serum.</th>
<th>Number of transformants in 1 ml. of culture</th>
<th>Activity of provoking factors derived from cultures of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provocation of competence tested on Streptococcus strains</td>
<td>Control without provoking factor</td>
<td>Challis</td>
</tr>
<tr>
<td>Challis</td>
<td>4 × 10^4</td>
<td>2 × 10^4</td>
</tr>
<tr>
<td>S. sanguis type I/II</td>
<td>5 × 10^4</td>
<td>12 × 10^4</td>
</tr>
<tr>
<td>3437/48</td>
<td>8 × 10^4</td>
<td>8 × 10^4</td>
</tr>
<tr>
<td>Wicky</td>
<td>0</td>
<td>88 × 10^6</td>
</tr>
<tr>
<td>E 91/46</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Channon</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blackborn</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hs</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. sanguis type I</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. sanguis type II</td>
<td>0</td>
<td>0</td>
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</table>

DISCUSSION

Experiments presented in this paper show in cultures of transformable streptococci the presence of an exocellular factor which seems to be essential for development of competence. This factor was never found in cultures which were not competent during a given period of growth. On adding competence-provoking factor, transformation of the strain Challis and Streptococcus sanguis type I/II was produced in medium ET3 in absence of serum or albumin. It seems clear, therefore, that serum or albumin are not essential for transformation of organisms rendered previously competent. In the absence of serum or albumin and provoking factor, no significant transformation was ever observed. It may be concluded that the presence of serum or albumin is essential for production of provoking factor and, subsequently, for development of competence.

We were not able to transform cells of Streptococcus sanguis type I/II in the absence of serum: in this case albumin is not a proper substitute for serum. It is probable that an additional substance, beside albumin, present in serum, plays some role in development of competence in cultures of S. sanguis.

The factor which provokes competence seems to be an enzyme. The induction of competence is time and temperature dependent and the factor itself is heat sensitive and does not pass through Visking dialysis tubing. If the factor is an
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enzyme, we are dealing with a complicated reaction in which the amount of substrate (the cocci) is increasing during its action. In our experience the addition of even large amounts of provoking factor does not result in conversion of more than about 15% of non-competent cocci into competent ones. It therefore seems likely that in a given period of growth, only a part of the population in a culture is able to react with the provoking factor. Synchronization of division in cultures by cooling before the addition of provoking factor, did not change remarkably the degree level of competence.

The specificity of action of provoking factor produced by the three transformable streptococcal strains was tested on closely related bacteria all of which, including Streptococcus sanguis type I/II, contain the C polysaccharide common to group H Streptococcus. In medium ST+serum, reciprocal provocation of competence was demonstrated in most instances (Table 2). However, some specificity of action is evident. Provocation by homologous factors results in production of more transformants than provocation by heterologous factor. The extension of such provocation tests to other streptococci and related bacteria, such as pneumococci, is necessary to determine the range of specificity of action of these factors. Preliminary experiments revealed the probable presence of competence-provoking factor in sterile supernatant fluids of competent cultures of the R36A pneumococcus strain. A similar factor is, probably, also present in competent cultures of Hemophilus influenzae, as one may conclude from the data of Goodgal & Herriott (1961). The present findings suggest that development of competence in streptococcal cultures requires the action of an enzyme. The question may, therefore, be asked: what is the substrate of this enzyme? This question cannot be answered until the provoking factor has been purified and its action investigated on some bacterial constituents. First of all, one has to look for it in the cell wall.

REFERENCES