JOURNAL OF BACTERIOLOGY, June 1973, p. 1198-1205
Vol. 114, No. 3

Early Events in Development of Streptococcal Competence

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Received for publication August 1973

Appropriately timed use of trypsin, which inactivates competence factor (CF), and chloramphenicol made feasible a separation and characterization of early events in the development of competence in group H streptococci. Step 1 is production of CF, which is inseparable in time from the concomitant release of free CF into the medium. The producing cells, which are noncompetent at the time, also accumulate cell-bound CF (CB-CF) from the onset of CF synthesis. In step 2, the released CF is adsorbed or taken up in a trypsin-insensitive state by the producing cells and is not destroyed as previously suggested. This occurs rapidly in a transformation-supporting (complete) medium. The rapid decline in free CF is concomitant with the rise in CB-CF, and a maximal increase in the latter does not occur in cultures exposed to trypsin, which inactivates any trypsin-accessible CF. The rapid increase in CB-CF (above trypsin-treated levels) leads to step 3, the induction of competence. All of these steps probably require protein synthesis, because each is inhibited by chloramphenicol. The data also indicate that only free CF that is subsequently adsorbed, and which thus leads to maximal levels of trypsin-insensitive CB-CF, is the effective inducer of competence in either CF-producing (Challis) or CF-nonproducing (Wicky) cultures. The processes induced by the newly bound CF are not fully understood, but certain new properties, previously described by others as indicating competence, were measured during the several steps of competence development. Cell aggregation at pH 2 appears to be related to CB-CF and can be shown before this bound CF has induced competence. The ability of cultures to autolyze maximally can be diminished by trypsin treatment of precompetent cells without affecting subsequent competence development as measured by transformation.

Competence for deoxyribonucleic acid (DNA)-mediated transformation in some strains of group H streptococci has been shown to be induced by a streptococcal cell product known as competence factor (CF), and this induction process is known to protein synthesis (9, 10, 14). A similar CF system has been described for pneumonococci (15, 17, 18). Thus this sort of CF, or its mode of action, must differ from competence factors described in other bacterial systems in which protein synthesis is not required for their action (2, 17).

Although the nature and some properties of streptococcal CF have been defined (6), its mode of action is relatively unknown. It was shown in our prior studies (6, 7) to be produced before competence development by cells not yet competent or (in certain media) by cells which never achieved competence, thus demonstrating the separability of its production from subsequent events in competence development. In the present study, events are further defined by the times at which they are or are not inhibited by proteolytic enzymes (which inactivate CF) and by chloramphenicol (CAP).

MATERIALS AND METHODS

Organisms. Strains Challis-6, SBE I/II, and Wicky of group H streptococci were used (7, 13). Strain Challis-6 was employed for CF production and for studies on its transformation. Strain SBE I/II, resistant to 2 mg of dihydrostreptomycin per ml, served as a source of DNA, and transformation to the streptomycin resistance was used throughout. Strain Wicky is a nontransformable strain which requires the addition of CF for transformation and, therefore, was used to titrate CF activity (7).

Media. The compositions of the chemically defined media were previously described (7). Synthetic medium MS6 was used for growth and transformation of Challis-6 cells, MS6-T was used for Challis transformation studies only, and MS6-F was used for CF production. MS6-F was modified by lowering the tyrosine content to 2 mg/liter, and MS6 was modified by lowering the KH_2PO_4 to 2.0 g/liter and K_2HPO_4 to 13.0 g/liter. Brain-heart infusion broth (Difco) reinforced with 2.5% heat-inactivated horse serum (BHI-HS) was used for growth and transformation of strain Wicky. It was also used for strain Challis transformation.

Procedure for CF production in MS6-F medium. Details of CF production and purification, and all other materials and methods used, were previously described (6, 7). CF isolated from MS6-F was designated CF-S and that from BHI-HS was designated CF-HS (7).

Determination of CF biological activity. CF biological activity was measured by induction of Wicky cells into the competent state as previously described (6, 7). Briefly, it is expressed as the logarithm of the number of transformants per milliliter obtained by mixing 0.1 ml of CF-containing fluid, after appropriate dilutions of concentrated samples, with a standard inoculum of Wicky cells (10^8 cells) in a 1-ml final volume. CF used in these studies was previously heated at 60 C for 20 min to inactivate any autolysin (12).

Procedure for transformation of strain Challis-6. Transformation of Challis cells was done in MS6, MS6-T, or BHI-HS media as previously described for MS6-T (7).

Aggregation of cells at pH 2.0. Cells washed in water were suspended in 0.01 N HCl (pH 2), or cell suspensions in water were adjusted to pH 2 by adding 0.1 N HCl (10).

Extraction of CB-CF. CB-CF was extracted as described by Dobrzanski et al. (5) by heating washed cells at 60 C for 10 min at pH 2 in 0.01 N HCl.

Cell autolysis. Lysis was measured spectrophotometrically as described by Ranhand et al. (13). The values reported represent the percentage of the initial optical density (OD) lost after 2 h of incubation.

RESULTS

CF production and release (step 1) and adsorption (step 2) by noncompetent Challis cells prior to competence development. We reported earlier that added glutamate is required for competence development but not for CF production (7). Thus, by controlling the amount of glutamate in our defined media, CF production in MS6-F (no glutamate) can be separated from competence development in MS6-T (glutamate added). We also showed that, in Challis cultures, free CF disappeared quickly from either media. In order to determine whether the rapid decline in free CF in both media was due to CF destruction or readsorption, we determined free CF and CB-CF in Challis cultures grown in either MS6-F or MS6-T. Free CF was determined directly and after its precipitation by $(NH_4)_2SO_4$ saturation of 1 liter of culture supernatant fluids for each incubation time tested. CB-CF was extracted as described in Materials and Methods from the cells obtained from 1 liter of the same culture for each time tested.

The results obtained in MS6-F (no glutamate) are shown in Fig. 1. Replicate samples were analyzed with and without trypsin (100 μg/ml) added at zero time. This concentration of trypsin inactivated CF, but had no effect on cell viability or growth. Figure 1 (curve e) shows, as previously reported (7), that cultures of Challis incubated in MS6-F (no glutamate) do not transform at any time tested (0 to 280 min); nevertheless, they produce and release CF (curve b). Also, as expected, no free CF was detectable in the cultures with trypsin added (curve d). Free CF was detected early, and maximal levels were found after about 60 min (step 1). After 100 min, the free CF level declined quickly, and after 180 min no significant amount was detected. Curve a shows that CB-CF levels rose with, but less rapidly than,

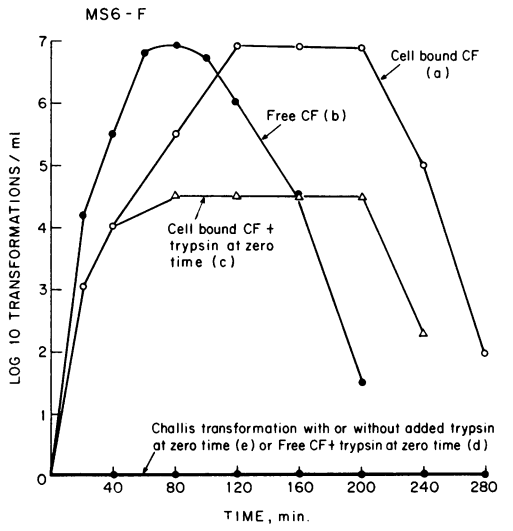

FIG. 1. *Free competence factor (CF), cell bound CF, and transformability of strain Challis cultures at different incubation times in 1 liter of MS6-F with and without added trypsin (100 μg/ml) at zero time. The biological activity of free CF (curves b and d) and CB-CF (curves a and c) is expressed as log_{10} strain Wicky transformants per milliliter. Strain Challis transformation (curve e) is expressed as log_{10} strain Challis transformants per milliliter after 20 min of incubation in fresh MS6-F. Procedures are detailed in Materials and Methods.*

rising levels of free (released) CF (curve b) and that a sharp decline in free CF was coincident with step 2 attainment of sustained, maximal levels of CB-CF (curve a). Furthermore, if free CF was destroyed by trypsin (curve d), no marked increase in CB-CF occurred (curve c) over the amounts of CB-CF which the cells retained from the onset of its synthesis.

The results obtained with Challis cultures in MS6-T (glutamate transformation medium) were similar, but differed in the times of attainment and duration of peak levels of both free and CB-CF (Fig. 2). Although both free CF (curve b) and CB-CF (curve a) were detectable at 20 min (as in MS6-F), high levels were achieved more rapidly (step 1). Rapid decline of free CF was again associated with attainment of maximal levels of CB-CF (step 2), and the latter was again prevented by trypsin (curve c). However, all events occurred earlier in incubation. Most significantly, the increase (above trypsin-treated levels) of CB-CF (curve a) was followed by the achievement of competence as measured by transformability of the CF-producing Challis cells (curve f), whereas trypsin at zero time completely prevented this transformation (curve e). That is, the peak of transformability

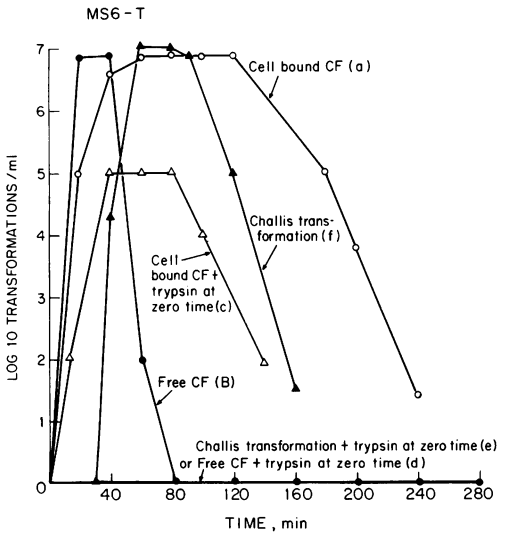

FIG. 2. *Free competence factor (CF), cell-bound CF, and transformability of strain Challis cultures at different incubation times in 1 liter of MS6-T with and without added trypsin (100 μg/ml) at zero time. The biological activity of free CF (curves b and d) and cell-bound CF (curves a and c) is expressed as $\log_{10}$ strain Wicky transformants per milliliter. Challis transformation (curves e and f) is expressed as $\log_{10}$ strain Challis transformants per milliliter after 20 min of incubation in fresh MS6-T. Procedures are detailed in Materials and Methods.*

coincided with attainment of maximal readsorbed CB-CF and not with peak levels of free CF. It seems that only released CF after its readsorption to cells in a complete medium is an effective inducer of competence. The fall in free CF and rise in CB-CF coincided, and maximal increase in the latter did not occur in trypsinized cultures (no free CF) in either MS6-F or MS6-T. This probably indicates that, normally, free CF is not destroyed as previously suggested (6) but, instead, is bound back to the producing Challis cells which retain it for several hours. Therefore, the maximal increases in CB-CF (curve a) are due to readsorption of free CF and not to new CB-CF which continues to be synthesized.

Although not shown in Fig. 1 and 2, addition of CAP (8 μg/ml) at zero time completely inhibited production of both free CF and CB-CF. Also, the amount of CB-CF extractable from Challis cells, incubated in MS6-F for 180 min or in MS6-T for 100 min, was not significantly affected by treatment of these cells with trypsin prior to the extraction of CF.

Table 1 presents more evidence that only the adsorption of free CF (step 2) in the absence of CAP leads to competence development (step 3) in CF-producing strain Challis in MS6-T. As indicated, trypsin, Pronase, or CAP were added at different times during incubation. The concentrations used did not affect cell viability. All the tubes were incubated for a total of 90 min before testing for Challis transformation in fresh MS6-T. All of the substances added to the cultures at zero time, or as late as 20 min of incubation, prevented transformation. When added at 40 min, incomplete inhibition occurred. At 60 min or later, there was no significant inhibition. Similar results were obtained in

TABLE 1. *Inhibition of challis transformation in MS6-T*

Additions to cultures (μg/ml)	Transformation of Challis cells after 90 min of incubation (incubation times in min before additions)[a]				
	0	20	40	60	90
None					5×10^6
Trypsin (5)	0	0	5×10^4	3×10^6	4×10^6
Pronase (1)	0	0	6×10^4	3×10^6	4×10^6
Chloramphenicol (7.5)	0	0	3×10^4	1×10^6	2×10^6

[a] At the indicated times the different compounds were each added to replicate 5-ml culture mixtures, and incubation continued for a total of 90 min before testing for Challis transformation in 1 ml of fresh MS6-T as described in Materials and Methods.

other transformation media as MS6 and BHI-HS. These results, in relation to those in Fig. 2, are interpreted as showing that free CF is progressively bound, during incubation, in protease-insensitive and active form to increasing numbers of cells and that protein synthesis is probably required throughout this period (step 2). Binding and competence induction are completed by 60 min (Fig. 2, curve a). Therefore, when free CF is destroyed by added proteolytic enzymes prior to its binding, or when all protein synthesis is inhibited by the addition of CAP before full competence is developed (60 min), transformability is inhibited. These results also show that addition of these proteolytic enzymes or CAP to fully competent cells (60 or 90 min) had no significant effect on transformability.

We recently reported that Challis cells transformed equally well in MS6, the growth medium, as in MS6-T, the transformation medium (7). That is, over 10% frequencies of transformation are obtained in either medium, but in MS6 only low amounts of free CF could be detected as compared with the amounts of free CF found in MS6-F or MS6-T (Fig. 1, 2). We therefore wanted to show whether in MS6, as in MS6-T, Challis cells produced, released, and readsorbed CF prior to competence development (Table 2). Challis cultures were grown in MS6 for 60 min, with or without added trypsin (100 μg/ml), to destroy any CF released. After 60 min of incubation, trypsin inhibitor (Sigma soybean; 100 μg/ml) was added to all samples, and incubation continued for 10 min. The cells then were tested for transformation with DNA with or without added, highly purified CF (step 3, CF-S as described in ref. 6). The transformation mixtures were incubated for an additional 20, 40, and 60 min before addition of deoxyribonuclease. In the control samples without trypsin, Challis cells (60-min old) were already fully competent and required only 20 min for DNA adsorption. Longer incubation with DNA (40 or 60 min) or with added, purified CF had no additional effect. On the other hand, Challis cells previously incubated in MS6 plus trypsin did not transform when exposed to DNA for 20, 40, or even 60 min in fresh MS6 and no trypsin. These same cultures transformed when exposed to added CF-S for 40 and 60 min. Clearly, a 20-min exposure to added CF and DNA was insufficient time for CF to induce competence in previously trypsinized Challis cells. This result indicates that trypsinized Challis cells (like Wicky cells which produce no CF) require added CF and a minimal period of 40 min of incubation after CF addition for competence induction. Inhibition of transformation by trypsin and restoration of transformation by CF added to trypsin-neutralized cultures indicate that free or trypsin-accessible CF must normally appear, even though transitorially, in MS6. It seems likely that in MS6, where Challis grows so quickly, free CF is released, adsorbed, and then lost so rapidly that little is therefore detectable at any one time in contrast to MS6-F or MS6-T, where these events do not occur so fast (7, 11).

TABLE 2. *Reversal of trypsin inhibition of Challis transformation in medium MS6 by addition of purified competence factor*[a]

Transformation time before addition of DNase (min)	Transformation of Challis cells (per ml)			
	No trypsin		Trypsin at zero time	
	No CF-S	CF-S added	No CF-S	CF-S added
20	1×10^7	1×10^7	0	0
40	2×10^7	1×10^7	0	3×10^6
60	1×10^7	1×10^7	0	2×10^7

[a] Challis cells were grown in medium MS6 for 60 min with and without added trypsin (100 μg/ml). Then trypsin inhibitor was added, and incubation continued for an additional 10 min. The cells were then tested for transformation with DNA and with or without added purified competence factor isolated from medium MS6-F (CF-S). Procedures are described in Materials and Methods.

CF adsorption by noncompetent cells. Because it is now apparent that both Challis and Wicky cells must adsorb free CF for competence development, we used Wicky cells in studies of CF adsorption and on properties of cells during competence development. Table 3 shows the effect of CAP on competence induction. Wicky cells were grown in BHI-HS for 50 min, which is the optimal physiologic age of Wicky cells for CF adsorption. The culture was divided into 50-ml replicate samples, and an excess of CF-S (7) was added to all samples (CF-HS may also be used [7]). The samples were then incubated, with CAP added at the times indicated (0 to 50 min) to stop competence induction by CF (Table 3, column 1). About 5 min after the addition of CAP (at each time tested), the cells were washed once in BHI-HS to remove free CF and CAP, and a fraction of the washed cells was tested for autolysis (prior to transformation), and another fraction of these cells was tested for transformation in triplicate samples by adding DNA plus nothing, trypsin, or CAP, respectively. The transformation mixtures were in-

TABLE 3. *Competence induction in Wicky cells*[a]

Induction period of Wicky cells with added CF-S			Transformations of washed Wicky cells per ml and additions to transformation mixtures		
Incubation times (min) before CAP additions	Properties of cells during induction[b]		None	Trypsin (100 μg/ml)	CAP (8 μg/ml)
	pH 2.0 aggregation	Cell lysis (%)			
0	2+	10	2×10^6	0	0
5	3+	10	4×10^6	2×10^6	0
10	4+	10	4×10^6	2×10^6	0
15	4+	50	3×10^6	4×10^6	1×10^5
30	4+	70	4×10^6	4×10^6	1×10^6
50	4+	85	5×10^6	4×10^6	5×10^6
50 (no CAP)[c]	4+	80	5×10^6	4×10^6	6×10^6

[a] Replicate samples of Wicky cells were grown for 50 min in brain-heart infusion broth reinforced with horse serum. Then an excess of purified competence factor isolated from medium MS6-F (CF-S) was added to each sample, and the mixture was incubated for an additional 50 min with chloramphenicol (CAP) added at the indicated times during this second incubation period. Then the cells from each indicated time were washed free of CF and CAP and tested for transformation in triplicate samples as indicated. Procedures are detailed in Materials and Methods.

[b] These properties of the cells during induction were measured as detailed in Materials and Methods.

[c] This control sample was incubated for 50 min without CAP, and then the cells were washed and tested for transformation as described in footnote *a*.

cubated for 90 min. Wicky cells (50-min old), incubated briefly with CF and CAP from zero time, adsorbed or carried over enough CF to transform them after an additional 90 min of incubation (Table 3, line 1, column 4); but the CF bound in the absence of protein synthesis was trypsin sensitive, as shown by the inhibition of transformation by trypsin (Table 3, line 1, column 5). In addition, these cells failed to transform in the presence of CAP (Table 3, line 1, column 6). These findings show that the CF was only loosely associated with the cells and that CAP inhibited its binding in trypsin-insensitive form and was therefore bound in a trypsin-insensitive state only during additional incubation in the absence of trypsin and CAP. However, only as short a period of uninterrupted protein synthesis as 5 min after exposure to CF was needed to adsorb it in a trypsin-insensitive state (Table 3, line 2, column 5). Nevertheless, these cells still failed to transform in the subsequent presence of CAP (Table 3, line 2, column 6) and, in fact, required more than 30 min of uninterrupted protein synthesis after exposure to CF to transform optimally in the later presence of CAP (Table 3, line 6, column 6). These results indicate at least two different steps in competence development of Wicky cells, which are inhibited by CAP and thus probably require protein synthesis: (i) a brief period for adsorption or binding of CF in a trypsin-insensitive state followed by (ii) induction by the CB-CF of later stages of competence achievement.

Reported to be indicative of the competence state are the cell aggregation at pH 2 (11) and a stimulated autolysis (13). We tested these properties during competence development in Wicky cells at the various indicated times. The pH 2-caused aggregation of Wicky cells increased as CB-CF (trypsin-insensitive) increased (Table 3, column 2), but this property was present prior to competence development (line 2 and 3, column 6). Similar results, not shown here, were obtained with Challis in MS6-F in which CF is made, released, and slowly adsorbed, although these cells never become competent and aggregate at pH 2. These results suggest that the aggregation of cells at pH 2 is a function of exposure to CF, the effect of which increases with time of exposure.

On the other hand, as first shown by Ranhand et al. (13), significant stimulated autolysis (Table 3, column 3) was detected as competence developed. Significant autolysis did not occur until after 15 min of initial protein synthesis, which also achieved some degree of competence. Maximal autolysis coincided with maximal transformability (Ranhand et al., Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 51, 1972); nevertheless, the possible roles of autolysin(s) in competence remains to be substantiated (13). Although autolysins may be required for competence, as measured by transformability, the measurable increase in autolysis observed after competence development may not be required (Table 4). Challis cells were grown in 100 ml of MS6, MS6-T, or BHI-HS for 60 min for competence development (samples 1 to 3); Wicky cells (sample 4) were grown in 100 ml of BHI-HS for 50 min, CF-S was added, and the mixtures were incubated for an additional 50 min to induce competence. The cultures of competent cells (samples 1 to 4) were divided into two 50-ml fractions and incubated for 30 min at 37 C with and without 100 μg of trypsin per ml. Then both trypsin-treated and -untreated cells were tested for transformation (without washing) and also for autolysis (13). The results show that competent Challis and Wicky cells, which normally show extensive lysis (over 70%), lost most of

TABLE 4. *Effects of trypsin on competent and noncompetent cells*

No.	Cells	Media used for competence development[a]	Transformation/ml		Autolysis (%)	
			No trypsin treatment	Trypsin treatment	No trypsin treatment	Trypsin treatment
1.	Challis-6[b]	MS6	4×10^7	4×10^7	75	12
2.	Challis-6[b]	MS6-T	1×10^7	1×10^7	80	10
3.	Challis-6[b]	BHI + HS	4×10^7	4×10^7	82	15
4.	Wicky[b]	BHI + HS	6×10^6	6×10^6	82	11
5.	Wicky[c]	BHI + HS		7×10^6	85	12

[a] See Materials and Methods for media descriptions.

[b] Competent cells were obtained as described in the text. Each sample was divided into two fractions. One fraction was treated with 100 μg of trypsin per ml for 30 min at 37 C. Untreated cells were similarly incubated. Then the cells were washed in saline and tested for transformation and autolysis as described in Materials and Methods. Controls of competent cells treated in a similar manner with trypsin previously inactivated with trypsin inhibitor, or treated with trypsin at 4 C or with heat-inactivated trypsin, were not affected.

[c] Trypsin (100 μg/ml) was added to noncompetent Wicky cells after only 5 min of exposure to competence factor, and incubation continued throughout the rest of the competence induction period (45 min) with the added trypsin. Other conditions were as described above and in the text.

their susceptibility to autolysis after trypsin treatment, but their transformability was not significantly affected. Even more significant are the results obtained in sample 5, which was treated with trypsin prior to competence development. Trypsin was added to 200 ml of a culture of noncompetent, 50-min-old Wicky cells after only 5 min of exposure to CF, instead of 50 min exposure as in sample 4, and incubation was continued throughout the rest of the CF competence induction period (45 min) with the added trypsin. Only 5 min of contact was required by Wicky cells to bind sufficient free CF for competence induction (Table 3, line 2, column 5). Samples (50 ml) were removed at the time of trypsin addition and after 10, 30, and 45 min of incubation with trypsin. The samples then were tested for transformation (without washing) and autolysis as described (13). Although only the results obtained after 50 min of incubation with CF and trypsin are shown in Table 4, it was found that Wicky cells developed optimal competence after a 30-min-induction period in the presence of trypsin, but no significant increase in autolysis (12 to 20%) was detected at any cell age tested (5, 10, 30, and 50 min). These results indicate that a detectable increase in autolysis, but probably not all, may be inhibited throughout competence development without affecting transformability.

Trypsin-accessible CF, either free or bound, was not required in the late stages of competence or for DNA uptake and/or recombination. Similar results were recently reported (8).

DISCUSSION

The established facts, that protein synthesis is required for competence induction by CF (9, 14, 15) and that CF is inactivated by proteolytic enzymes (6, 17), prompted the use of CAP and of trypsin in the present study to further define the events leading to competence development by the times at which they are or are not inhibited by either CAP or trypsin. The CAP inhibition observed has been assumed to be due mainly to protein synthesis inhibition, although other possible effects of CAP have not been ruled out. Nevertheless, the CAP and trypsin effects presented here have permitted the division of competence development into the following sequential events: (i) CF production by noncompetent cells and the concomitant release of free CF into the medium, although cells retain CB-CF from the onset of CF synthesis; (ii) the released CF is bound (or readsorbed) by the producing cells and most, if not all, is not destroyed as previously suggested (7). The rapid adsorption of free CF into a trypsin-insensitive state seems to require protein synthesis, for CAP inhibits it and requires a complete medium (7). The rapid decline in free CF is concomitant with the rise in CB-CF, and a maximum increase in the latter does not occur in cultures treated with trypsin, which destroys any CF accessible to trypsin. (iii) The rapid increase in CB-CF (above trypsin-treated levels) leads to the achievement of competence if a third period of protein synthesis (over 30 min) is allowed to take place (9, 14, 15, 17). The results presented indicate that only released CF after its adsorption to cells in a complete medium (which leads to the maximum levels of CB-CF) is an effective inducer of competence.

The finding that only adsorption (binding) leads to competence induction and develop-

ment in both CF-producing (Challis) and CF-nonproducing (Wicky) cells suggests that the initial role of this positively charged and extremely basic product (6) may be to alter the negatively charged cell wall. This cell wall modification by the newly bound CF would make the cell more receptive to the reversible binding of added DNA. It may also account for the aggregation at pH 2.0 of cells not yet competent, but containing CB-CF, and for the decreased ability of the cells to bind methylene blue (11). Although these induction processes by the newly CB-CF are not fully understood, certain new properties previously described as indicating competence can be measured, such as an increase in autolysis susceptibility (13), cell aggregation at pH 2.0 (11), and a requirement for new protein synthesis (9, 14) which finally results in DNA uptake and recombination as measured by transformation.

Pakula et al. (11) found that only competent cells aggregate at pH 2.0, but our present findings suggest that aggregation of cells at pH 2.0 is related to trypsin-insensitive CB-CF which appears before competence is achieved. It is not, therefore, under our test conditions, an absolute measure of competence; nevertheless, all competent cells aggregate at pH 2.0, and these cells retain this property for several hours after the short period of transformability has ended.

Also, autolysis susceptibility was reported by Ranhand et al. (13) to coincide only with maximal transformability. However, our findings (Table 4) show that trypsinization of cultures already competent or of cultures prior to competence development (but after CF adsorption) results in the loss of the significant increase in cell susceptibility to autolyse in lysing buffer (13) without affecting transformability. Conversely, recent results from our laboratory show that lysogenized cultures which exhibit maximal autolysis susceptibility, nevertheless do not transform because they fail to bind DNA (C. L. Parsons et al., Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 50, 1972). It therefore appears that although increased autolysis susceptibility may normally indicate attainment of a certain stage of competence, it is not an absolute measure of transformability. Although these results do not negate the possible involvement of low levels of autolysin in competence development (13; J. M. Ranhand et al., Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 51, 1972), its possible role remains to be elucidated.

Another aspect of step 3 is the appearance of new proteins (14). It is possible that a CF-membrane protein complex could function to bind added DNA irreversibly and that such protein results from induction by CF. A protein which can bind to DNA, and is involved in its replication and recombination, has been reported (3). This regulatory product (of a known bacteriophage gene) may control cellular processes at the membrane level, other than those concerned with DNA. However, there is no evidence for such a regulatory protein appearing in the streptococcal system as a result of induction by CF, although a DNA-reactive factor (IF) has been found in streptococci capable of becoming competent (8). On the other hand, CF itself is a highly basic and positively charged substance which is probably protamine-like (6), and other protamine-like products are reported to act as regulators in higher organisms (D. M. Lam and W. R. Bruce, Fed. Proc., **30**:1086, 1971; ref. 1, 4). It is therefore possible that CF, in its performance as an activator or hormone-like cell product, as suggested by Tomasz (16), may function in normally undetectable amounts to regulate cellular metabolism at the membrane level and that the induction of competence in certain strains (otherwise properly conditioned and exposed to high CF levels) may not be the CF principal function.

ACKNOWLEDGMENTS

We thank David Brand and Catherine Dixon for their excellent technical assistance. We also thank Arthur Schade for very helpful discussions.

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