MARKED ENHANCEMENT OF DIPLOCOCCUS PNEUMONIAE COMPETENCE FOR TRANSFORMATION BY TRYPTIC PEPTIDES OF CASEIN

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Abstract

Competence development in cultures of Diplococcus pneumoniae is markedly different in the presence of tryptic peptides of casein. Three effects are discernible following the addition of tryptic peptides; a change to a less complex pattern of competence, a shift in the peak competence range to a cell concentration which is 20- to 25-fold higher and a 10-fold increase in the overall percentage of competent cells.

Studies involving the expression of new biochemical properties following the introduction of genetic material during DNA-mediated transformation are limited by the inability to obtain adequately high levels of competence (transformability) in recipient cultures. In Diplococcus pneumoniae, the expression following transformation of only qualitative biochemical changes has been studied (1). Similar experiments measuring the expression of quantitative biochemical changes appeared desirable in connection with studies (2-5) in our own laboratory on the dihydrofolate reductase activities in this organism.

During studies on the effect of a variety of nutritional factors on the development of competence in D. pneumoniae, we were able to enhance the level of competence 10-fold by supplementating the growth medium with tryptic casein peptides. Of equal importance was the altered pattern of competence also observed in these cultures. This enabled us to obtain this enhancement at a competence peak which occurred at a cell concentration 20- to 25-fold higher than in cultures without tryptic peptides.

Materials and Methods

Both the wild-type R6 recipient and a streptomycin (str) resistant donor strain of D. pneumoniae were obtained from Dr. R.D. Hotchkiss, Rockefeller University. The str mutation in this strain is transformed with moderately high efficiency (2). DNA was prepared by a modification (6) of the method of Mayers and Spizizen (7). Competent cultures were exposed to saturating concentrations of DNA (10-50 μg/ml) at 30°C. Maximum transformation occurred in 30 min at pH 7.9 following a 1/20 dilution of culture in a solution containing 50 mM K_2HPO_4, 2.5 mM $MgCl_2$, 0.45 mM $CaCl_2$, 11 mM glucose and 0.167 mM cystine. Transformed cultures (2 ml) were treated with 5 μg of deoxyribonuclease (Worthington), diluted 1/20 in a semisynthetic casein hydrolysate medium (8) and incubated for 75 min at 37°C for expression of the str property. The assay for str transformant colonies and total colony forming units have been described (8). The total viable cell count is calculated as the product of the total colony count and 2.78, the average chain length in this growth medium as determined microscopically. A calculation for percentage transformation is based on the total cell count at the time of DNA addition. No appreciable growth occurred during the period of exposure to DNA in the supplemented buffer solution.

The basic competence medium used during these studies was a somewhat improved version of the modification of the Adams and Roe (9) medium described earlier (8). It contains, per liter, 0.5 g vitamin-free acid hydrolyzed casein (Difco), 2 g sodium acetate, 2 g glucose, 40 mg L-cystine, 6 mg L-tryptophane, 6 μg biotin, 0.6 mg nicotinic acid, 0.6 mg thiamine.HCl, 0.28 mg riboflavin, 50 mg asparagine, 20 mg choline chloride, 200 mg sodium thioglycollate, 0.5 mg $FeSO_4.7H_2O$, 0.5 mg $CuSO_4.5H_2O$, 0.5 mg $ZnSO_4.7H_2O$, 0.4 mg $MnCl_2.4H_2O$, 10 mg $CaCl_2$, 8.7 g K_2HPO_4, 172 mg $NaHCO_3$, 8 gm bovine serum albumin fraction V (Armour) and 3% of a fresh yeast extract preparation in water. For best results, this medium was

made up fresh daily. A solution containing the casein hydrolysate, sodium acetate, potassium phosphate, cystine and tryptophane was adjusted to pH 7.4 and sterilized by boiling for 10 min. After cooling, the other ingredients were added as sterile supplements prepared on the same day whenever possible. The medium was then brought to volume with sterile distilled H_2O.

A tryptic digest of casein was prepared in our laboratory according to instructions provided by General Biochemicals Inc. One hundred and twenty five g of Vitamin-Free test casein (GBI) were suspended in 1 L distilled H_2O at 80°C. A solution of Na_2CO_3 (6.25 g in 250 ml distilled H_2O) was heated to 80°C and added while vigorously shaking. After stirring for 2-3 hours, the suspension was cooled to room temperature and 1 g of trypsin 1:250 (GBI) in 4 ml distilled H_2O added. Toluene was layered on the surface and the suspension kept at 37°C for 20 hours. The digest is filtered through several layers of cheese cloth until clear and the pH adjusted to 6 with acetic acid. Fifty g of charcoal (Darco G-60) was added and the preparation stirred for 30 min. The solution was filtered, adjusted to pH 4.5 with acetic acid and another 50 g of Darco G-60 added followed by stirring for 30 min. The charcoal was removed by filtration, the pH adjusted to 7.0 and the solution sterilized by autoclaving. Digests obtained from Nutritional Biochemical Corp. were assumed to be prepared by a similar procedure. The concentration of peptides in each hydrolysate was determined by dry weight corrected for the amount of other ingredients.

Results and Discussion

The development of competence in D. pneumoniae cultures is shown in Figure 1. Ten ml of medium were inoculated with 1 to 2×10^7 cells from a freshly grown logarithmic phase culture and incubated at 37°C. Growth was monitored by optical density (O.D.) and samples were removed from each culture at varying intervals to be tested for transformability. The competence pattern observed in the basic competence medium without tryptic

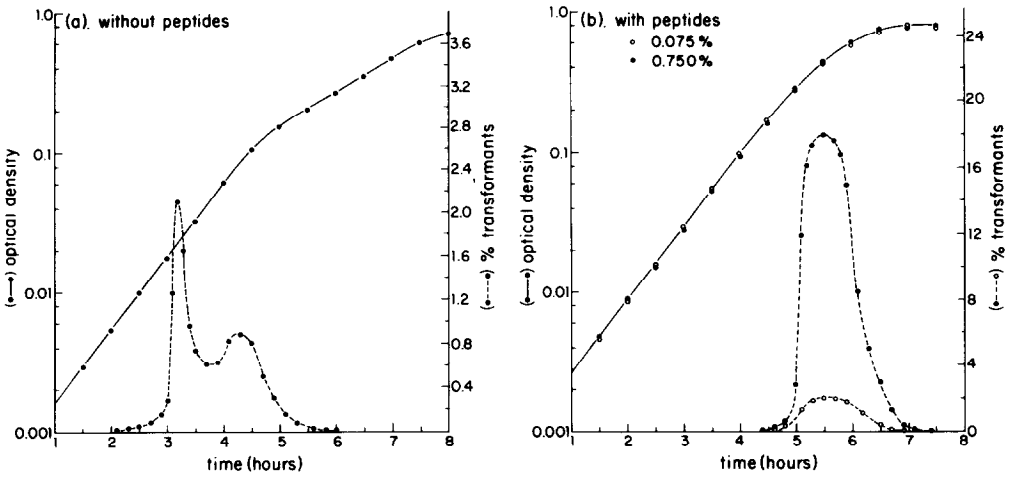

Figure 1. The development of competence by D. pneumoniae during growth in the presence and absence of tryptic peptides. Initial cell conc. = 1 to $2x10^6$ cells/ml. Samples were removed at varying intervals of time and cells transformed as described in the Text.

peptides exhibits two peaks. A very sharp peak first occurs within an O.D. range of 0.02 to 0.03 (4.4 to $6.6x10^7$ cells/ml). The level of transformability at this peak may reach 2.0 to 2.5% under optimum condition. A broader peak occurs in the vicinity of O.D. 0.1 ($2.2x10^8$ cells/ml) with the level of transformability seldom reaching 1%. This pattern of competence in this medium has been observed in our laboratory ever since it was first described a number of years ago (10). The competence pattern in cultures grown in the presence of tryptic peptides is strikingly different. Competence occurs as a single, but much broader, peak at O.D. 0.4 to 0.5 (8.8 to $11.0x10^8$ cells/ml). The level of transformability obtained with as little as 0.075% peptides was 2%. With 0.75%, the level approached 20%.

The effect of varying concentrations of tryptic peptides on competence development is more clearly shown in Figure 2. Competence at each concentration still occurs within an O.D. range of 0.3 to 0.6. The level of competence observed is a linear response to an increase in concentration

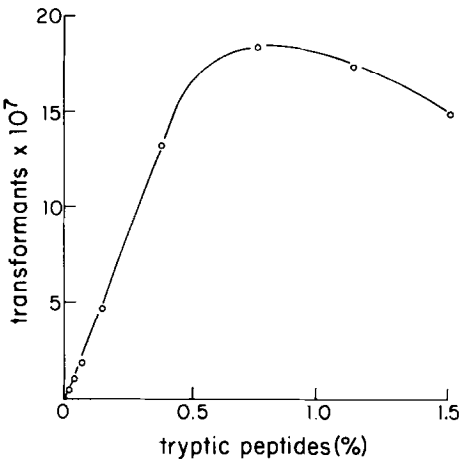

Figure 2. The effect of tryptic peptide concentration on the development of competence by D. pneumoniae. The conditions of the experiment were the same as those described in the text and in the legend for Figure 1. The concentration of tryptic peptides is based on a dry weight determination on the liquid hydrolysate.

to about 0.5%. The maximum level of competence occurred at a concentration of 0.75% with a diminution of competence seen at higher concentrations.

A similar effect on D. pneumoniae competence was obtained with three different tryptic digests, two of which were obtained from commercial sources. The results of this comparison are seen in Table 1. The tryptic digest prepared in the author's laboratory was also subjected to exhaustive dialysis against distilled water and then tested for competence enhancement. The dialyzed digest was equally as effective (Table 1) as the original material in increasing the level of competence. Ingredients of the digest other than casein peptide were tested [see Table 1, control (a)] and found not to enhance competence. An extract of unhydrolyzed casein [see Table 1, control (b)] was also tested for competence enhancement, but none was detected. The competence pattern obtained in the presence of these two control preparations was indistinguishable from that obtained with the basic competence medium alone. These results would appear to associate competence enhancement with the presence of the tryptic peptides themselves rather than some other constituent of the digest.

Table 1. Competence production in the presence and absence of tryptic casein peptides[1]

Experiment	Supplement	Transformants/ml x 10^6 (cell no. = 4.4×10^7/ml)[2]	(cell no. = 8.8×10^8/ml)[2]
I	-	0.084 (1.9%)	0
	digest A[3]	0	140 (15.9%)
	digest B[4]	0	153 (17.3%)
	digest C[5]	0	125 (14.2%)
II	-	0.071 (1.6%)	0
	digest C	0	107 (12.2%)
	dialyzed digest C	0	123 (13.9%)
III	-	0.054 (1.2%)	0
	digest C	0	171 (19.1%)
	control (a)[6]	0.088 (2.0%)	0
	control (b)[7]	0.080 (1.8%)	0

[1]Experimental conditions are described in the text and legend for Figure 1.
[2]Cell concentration at which maximum competence occurs.
[3]Liquid tryptic digest obtained from General Biochemical Corporation (final conc. = 0.75%).
[4]Liquid tryptic digest obtained from Nutritional Biochemical Corporation (final conc. = 0.75%).
[5]Liquid tryptic digest prepared in author's laboratory (see Methods section), (final conc. = 0.75%).
[6]A control solution carried through the same procedure used for preparing hydrolysate C, but without casein.
[7]A control solution carried through the same procedure used for preparing hydrolysate C, but without trypsin.

Two effects of tryptic peptides on competence are discernible from these experiments. The first is a shift in the range of maximum competence to a higher cell concentration. Only very low levels of peptides appear to be required for this shift. The second effect is on the absolute level of competence obtained. While an alteration in the competence pattern was observed with as little as 0.0375% tryptic peptides, twenty times this amount was required for maximum competence to develop. The alteration of the competence pattern might be only indirect, i.e. a

result of a nutritional effect on growth in this medium. Growth in the presence and absence of tryptic peptides is in fact somewhat different (Figure 1). In the latter case, the culture assumes a slower, but still constant, rate of growth above O.D. 0.1, suggesting a nutritional depletion at this point. When peptides are added, even at the lowest concentration tested (0.0375%), the culture does not exhibit this bimodal form of growth.

The complexity of the competence process in D. pneumoniae as well as in other bacterial species is well documented (11). The relavence of these findings to this process must await further work. However, these findings, are of some practical importance for workers utilizing this transformation system and, in illustrating that competence is a nutritionally manipulative process, provide a possible experimental approach to other studies in this area.

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References

(1) Lacks, S. and R. D. Hotchkiss Biochem. and Biophys. Acta 45, 155 (1960).
(2) Sirotnak, F. M. and S. L. Hachtel Genetics 61, 293 (1969).
(3) Sirotnak, F. M., W. A. Williams and S. L. Hachtel Genetics 61, 312 (1969).
(4) Sirotnak, F. M. Genetics 65, 391 (1970).
(5) Sirotnak, F. M. J. Bacteriol. 106, 318 (1971).
(6) Sirotnak, F. M., R. B. Lunt and D. J. Hutchison Genetics 49, 439 (1964).
(7) Mayers, V. L. and J. Spizizen J. Biol. Chem. 210, 877 (1954).
(8) Sirotnak, F. M., R. B. Lunt and D. J. Hutchison J. Bacteriol. 80, 648 (1960).
(9) Adams, M. H. and A. S. Roe J. Bacteriol. 49, 401 (1945).
(10) Sirotnak, F. M. and D. J. Hutchison Bacteriol. Proc. p. 36 (1959).
(11) Tomasz, A. Ann. Rev. Genetics 3, 217 (1969).