Surface proteins in the transduction of groups A and G streptococci

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Summary. Four pairs of M+SOR+ and M–SOR– variants of group-A type-49 streptococci were compared as receptor strains in transduction of a streptomycin-resistance marker. The yield of transductants was 5–9-fold greater with the M–SOR– variants than with the corresponding M+SOR+ variants. Treatment of M+SOR+ variants of type-49 streptococci with trypsin enhanced the rate of transduction by 16–35-fold whereas trypsin treatment of corresponding M–SOR– variants resulted in minimal enhancement (5-fold or less). With trypsin treatment the numbers of transductants were approximately equal in pairs of M+SOR+ and M–SOR– variants.

Enhanced transduction (10–26-fold) of streptomycin resistance was obtained by trypsin treatment of another seven M+SOR+ type-49 strains, of diverse phage subtypes and from various geographical locations. A wide range of enhancement (5–46-fold) was found in eight of nine M+ strains of group-A type-6 streptococci. With trypsin treatment, three of 10 transducible group-G strains showed enhanced transduction (10–13-fold) of a plasmid containing a determinant for erythromycin resistance.

Transductional enhancement is proteolytic in nature, being enhanced by trypsin, chymotrypsin, papain, pronase and streptococcal proteinase. Although interference with phage adsorption by surface proteins would appear to be the most obvious explanation for these findings, further studies are required to define more clearly the mechanism of trypsin enhancement.

Introduction

The genetic determinants for various markers such as antibiotic resistance, resistance to UV light, production of streptolysin S, and bacteriocin production have been transduced by bacteriophage in group-A streptococci (Wannamaker, 1982). The peptidoglycan receptor site for the A25 virulent bacteriophage and the mechanism of attachment have also been described (Cleary and Johnson, 1977).

The factors that influence the frequency of transduction are not well understood. This study addresses the question of the influence of surface protein(s) of recipient streptococci of groups A and G on the yield of transductants.

Materials and Methods

Test strains

Most of the T14/49, M type-49 strains of group-A streptococci have been described in previous studies (Skjold and Wannamaker, 1976; Skjold *et al.*, 1982). Additional strains of this serotype were isolated in Great Britain (Fraser *et al.*, 1979; Slack *et al.*, 1982). The group-A, M type-6 and the group-G strains were obtained from a wide variety of geographical sources. Three group-A strains were used as donors: two streptomycin-resistant strains (M49 strain GT-9278 Srt^r and M6 strain Gt-9440 Str^r) and a strain with a plasmid marker for erythromycin resistance (M6 strain, GT-9440ERL1).

All strains of group-A streptococci and their variants were classified according to M and T antigens by standard methods (Williams, 1958). Phage typing of type-49 strains was performed as previously described (Skjold and Wannamaker, 1976; Skjold *et al.*, 1982). The serum opacity reaction (SOR) was examined in broth culture supernate and in hot-acid extracts of the cells (Widdowson *et al.*, 1970). Plates of Islam medium (Islam, 1977) containing hyaluronidase 68 µg/ml (Sigma) (McCarty,

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1966) were used to distinguish opaque from translucent colony variants.

Enzyme treatment

Unless stated otherwise, streptococcal cells were digested for 1 h at 37°C with trypsin (1 × crystallised, Sigma) 25 mg/ml in No. 1 broth (Wannamaker *et al.*, 1973) for transduction. A highly purified preparation of the zymogen of streptococcal proteinase was kindly provided by Dr D. T. Liu. For activation it was flashed with nitrogen and incubated at 37°C for 30 min at a concentration of 1.27 mg/ml in 0.067 M phosphate buffer (pH 7.6) containing 0.001 M ethylene-diaminetetra-acetic acid and 0.0005 M dithiothreitol.

Phage-associated lysin was prepared in the laboratory by propagation of the C-1 virulent phage on the group-C streptococcus Azgazardah strain GT-9314 (Maxted, 1957).

Other enzymes used in these experiments were: α chymotrypsin (3 × crystallised), TPCK trypsin, TLCK chymotrypsin, pepsin and papain (all from Sigma). Pronase was obtained from Calbiochem-Behring, PO Box 12087, San Diego CA 92112.

Ovomucoid and soybean inhibitor were both obtained from Sigma.

Transduction was performed with phage A25TS1-2 by methods previously described (Skjold *et al.*, 1982). For detection of erythromycin-resistant transductants, erythromycin (Sigma) 20 µg/ml was used in the top layer of the plates. Transduction of group-G strains required an adjustment of the transduction mixture to 0.4 M NaCl.

Results

Pairs of opaque (M+SOR+) and translucent (M−SOR−) colony variants from four strains of

type-49 streptococci isolated in Great Britain were selected and purified on Islam agar plates. Subcultures of these variant colony pairs were compared as recipients in transduction of a marker for streptomycin resistance. The yield of transductants was 5–9-fold greater with the M−SOR− variants than with the corresponding M+SOR+ variants (table I).

The possibility that the presence of protein antigens at or near the surface of recipient cells might serve as a barrier to efficient transduction was explored by treating recipient cells with proteolytic enzymes. Trypsin treatment of M+SOR+ recipients resulted in a 16–35-fold increase of transductants whereas trypsin treatment of their corresponding M−SOR− variants resulted in a minimal (3–5-fold) enhancement in the yield of transductants (table I). In preparations treated with trypsin the yield of transductants was roughly equal in pairs of M+SOR+ and M−SOR− recipients. Lancefield hot HCl extracts of trypsin-treated M+SOR+ cells revealed no trace of residual M or SOR proteins.

We did not obtain stable translucent variants that consistently correlated with the absence of M and SOR proteins in studies with seven other opaque M+SOR+ type-49 strains isolated in other geographical areas. When trypsin-treated M+SOR+ strains were used as recipients, the yield of transductants was 10–26 times greater than that obtained with non-trypsinised cells from the same strains (table II).

The number of transductants increased in proportion to the logarithm of the trypsin concentra-

Table I. The effect of trypsin on transduction of a streptomycin-resistance marker to paired M+SOR+ and M−SOR− variants of group-A type-49 strains*

Recipient strain no.†	Phage subtype	Colony morphology	Serological classification		Serum opacity reaction	Number of transductants		Fold increase or decrease with trypsin
			T	M		without trypsin	with trypsin	
80–220	II	Opaque	14/49	49	+	13	376	+29
		Blue	14/49	NT	—	116	418	+4
81–081	II	Opaque	14/49	49	+	12	415	+35
		Blue	14/49	NT	—	58	300	+5
77–396	VI‡	Opaque	14/49	49	+	24	382	+16
		Blue	14/49	NT	—	170	444	+3
81–071	VI	Opaque	14/49	49	+	13	330	+25
		Blue	14/49	NT	—	116	534	+5

NT = Non-typable.

* Donor strain was a streptomycin-resistant variant of an M type-49 streptococcus (GT-9278 Str^r).

† University of Minnesota.

‡ New provisional phage subtype.

Table II. The effect of trypsin on transduction of a streptomycin-resistance marker to other M+SOR+ group-A type-49 streptococcal strains*

Recipient strain no.†	Geographic location	Phage subtype	Number of transductants		Fold increase or decrease with trypsin
			without trypsin	with trypsin	
GT-8760	Red Lake	I	145	2640	+18
Gt-6479	Alabama	I	108	2784	+26
GT-6266	Chile	II	24	449	+19
GT-7907	Netherlands	II	87	893	+10
GT-6481	Alabama	V	180	3360	+19
GT-6256	Czechoslovakia	VI‡	186	4896	+26
GT-7906	Czechoslovakia	VI	233	4032	+17

* Donor strain was a streptomycin resistant variant of an M type-49 streptococcus (GT-9278 Str).

† University of Minnesota.

‡ New provisional phage subtype.

tion, over a trypsin concentration range of 0.1–40 mg/ml (figure). With most strains, concentrations of trypsin beyond 40 mg/ml resulted in a levelling off or a decline in the yield of transductants (data not shown). Digestion periods of longer than 1 h did not result in significant enhancement of transduction, and overnight digestion resulted in a definite decrease in the enhancing effect. Best results were obtained when the trypsin-digested culture was added directly to the transducing phage suspension, i.e., without washing or adjusting the pH or changing the suspending medium.

To rule out the possibility that trypsin may have acted on the phage rather than the streptococci,

phages were treated with trypsin which was subsequently inactivated with soybean inhibitor. As there was no enhancement of transduction when the phage was pre-treated with trypsin in this way, proteolytic action on the phage did not account for the transduction enhancement that we report. When the purer 2 × crystallised trypsin (Sigma) was compared with the standard 1 × crystallised trypsin, the enhancement effect was essentially the same at equal concentrations of the two preparations over the range 0.1–57 mg/ml. This indicated that impurities in the trypsin were probably not involved in the effect on transduction.

To determine whether enhanced transduction was specific for trypsin or depended on proteolysis in general, other proteolytic enzymes were tested (table III). Chymotrypsin was tested over the range 0.1–40 mg/ml and had activity similar to that of trypsin. To eliminate the possibility that the chymotrypsin activity of the trypsin enzyme or the trypsin activity of the chymotrypsin enzyme was responsible for transduction enhancement, TLCK chymotrypsin (free of trypsin activity) and TPCK trypsin (free of chymotrypsin activity) were tested and found to have the same activity as trypsin and chymotrypsin. The activity of trypsin and chymotrypsin was inhibited by pretreatment with soybean inhibitor 40 mg/ml and ovomucoid 80 mg/ml for 1 h at 37°C.

A 2-fold enhancement of transduction was obtained with pepsin 3 mg/ml at pH 5.5; pepsin was tested over the concentration range 0.1–66 mg/ml. Pepsin treatment at a lower pH inhibited transduction. Papain and pronase each enhanced transduc-

Figure – The effects of variations in trypsin concentration on the yield of streptomycin-resistant transductants of an M+SOR+ variant of a type-49 streptococcus (81-071 opaque). The yield of transductants in the control population (without trypsin treatment) for this experiment was 107 (not shown in figure).

Table III. The effect of proteolytic enzymes on transduction of a streptomycin-resistance marker to an M + SOR + group-A, type-49 streptococcus 81-071*

Enzyme	Enzyme range mg/ml	Optimum enzyme concentration mg/ml	Fold increase at optimum enzyme concentration
Trypsin	0.1-40	40	+20
(TPCK) trypsin†	0.1-40	40	+21
Chymotrypsin	0.1-40	40	+21
(TLCK) chymotrypsin‡	0.1-40	40	+18
Strep proteinase	0.2-0.88	0.88	+6
Pronase	0.1-10	1	+5
Papain	0.1-10	1	+5
Pepsin	0.1-66	3	+2

* Donor strain was a streptomycin-resistant variant of an M type-49 streptococcus (GT-9278 Str^r).

† Free of chymotrypsin activity.

‡ Free of trypsin activity.

tion 5-fold. The optimal concentration for each enzyme tested over the range 0.1-10 mg/ml was 1 mg/ml. No enhancement occurred when phage-associated lysin was tested under optimal or suboptimal lytic conditions, with or without appropriate osmotic conditions necessary for maintaining cellular integrity.

Streptococcal proteinase at a concentration of 0.88 mg/ml was used to digest the cells and gave 3-fold enhancement of transduction after digestion

for 1 h and a 6-fold increase after 4 h. The proteinase was tested in concentrations of 0.2-0.88 mg/ml. Tests with higher concentrations were not done because supplies of the enzyme were limited.

Nine M + SOR - type-6 strains were also examined (table IV). In all except one of these strains, treatment with trypsin resulted in significant (5-fold or greater) enhancement of transduction of the streptomycin-resistance marker. Attempts to select M - variants of type-6 strains by their appearance

Table IV. The effect of trypsin on transduction of a streptomycin-resistance marker to M + group-A type-6 streptococci*

Recipient strain no.†	No. of transductants		Fold increase or decrease with trypsin
	without trypsin	with trypsin	
71-211	320	2880	+9
GT-9440	252	1296	+5
70-073	152	2160	+14
80-095	812	8491	+10
75-251	377	36	-10
74-494	731	4594	+6
71-681	46	2112	+46
80-393	125	1670	+13
79-065	143	6125	+43

* Donor strain was a streptomycin-resistant variant of an M type-6 streptococcus (GT-9440 Str^r).

† University of Minnesota.

Table V. The effect of trypsin on transduction of an erythromycin-resistance plasmid marker to group-G streptococci*

Recipient strain no.†	Number of transductants		Fold increase or decrease with trypsin
	without trypsin	with trypsin	
74-350	120	1537	+13
74-472	300	1032	+3
74-317	942	972	0
74-449	10	13	0
74-262	754	1160	+2
74-470	5	52	+10
P-11092A	638	797	0
70-165	60	132	+2
74-530	29	129	+4
74-411 (opaque)	246	2460	+10
74-411 (translucent)	1800	2460	0

* Donor strain was an M type-6 streptococcus (GT-9440ERL1) containing a plasmid (ERL1) which determines erythromycin resistance.

† University of Minnesota.

as translucent colonies on Islam plates were not successful.

Trypsin enhancement of transduction was less consistent with group-G streptococci (table V) than with group-A streptococci. In studies with 10 transducible group-G strains, enhancement of erythromycin resistance (10–13-fold) was found with only three strains. Opaque and translucent variants of one strain (74–411) were examined for their ability to grow in rotating tubes of blood from a normal human donor. The opaque variant multiplied (52-fold) whereas the translucent variant did not. These results indicated that colony morphology and functional behaviour of these variants corresponds to that of M+ and M– strains of group-A streptococci. This pair of variants also resembled group-A strains with respect to their behaviour as recipients in transduction experiments (table V).

Discussion

The occurrence of opaque colony variants of group-A streptococci was described by Griffith (1934) who found them valuable in studying surface-protein antigens. Gooder and Maxted (1961) suggested that the opaque colony appearance may indicate production of a large amount of M protein. In the present studies, we have found the selection of opaque and translucent colonies useful in the identification of M+ and M– variants, respectively, of type-49 streptococci isolated in Great Britain, but we have been generally unsuccessful with this approach in distinguishing variants of type-6 streptococci and type-49 streptococci isolated from other geographical locations. This latter observation is in accord with the discrepancies reported by Wilson (1959) and by McCarty (1966). McCarty (1966) and Swanson and McCarty (1969) correlated the opaque character with peculiarities in chain length and in cell wall septa.

Earlier workers have examined the effects of surface proteins of group-A streptococci on the ability of phages to adsorb to or lyse these bacteria. In studies with living group-A streptococcal cells, Friend and Slade (1966) reported that streptococci adsorbed phage just as efficiently after trypsin treatment as did an untreated sample. Fischetti and Zabriskie (1968), who also used living cells, detected no alteration in phage adsorption by group-A cells that had been treated with trypsin, chymotrypsin or papain. On the other hand, Cleary and Johnson (1977) showed that heat-killed cells of mutants producing large amounts of M protein adsorbed phage poorly, a finding that was reversed by trypsin treatment. In studies of a pair of M+ and M–

variants (Maxted, 1955) lawns of M+ variants were more resistant to phage infection than lawns of the M– variants. When Maxted grew these variants in broth containing trypsin and repeated the phage titration on trypsin-containing plates, he found no difference in the titres obtained with the M+ and the M– variants. In contrast, in a non-quantitative survey of stored strains of group-A streptococci, Krause (1957) found no difference between the frequency of phage lysis of strains that had retained M protein and those in which M protein was no longer detectable. Some of these differences reported by various authors may be attributable to the use of living or heat-killed cells, to the media employed, to differences in the smoothness of M+ and M– lawns, to use of paired and unpaired variants, or to other variables. Our findings and those of Maxted (1955) suggest that continued exposure of viable streptococci to relatively large amounts of trypsin may be an important factor for optimal demonstration of differences between M+ and M– variants, as trypsin-treated living cells may rapidly regenerate M protein (Fox and Kramnitz, 1956).

In view of these apparently conflicting observations, it is perhaps surprising that there is such a distinct difference in the number of transductants obtained with M+SOR+ and M–SOR– variants and that trypsin is so effective in enhancing the transduction of M+ strains of group-A streptococci.

Transduction enhancement with trypsin, chymotrypsin and to a lesser extent with the other proteolytic enzymes appears to be proteolytic in nature. The reason for the higher than normal concentration of the enzymes is not clear. Working with living streptococci under conditions optimal for transduction or the possibility of nonspecific hydrolysis (Kiel, 1971) may have contributed to the need for elevated enzyme concentration.

The relatively feeble enhancement of transduction found with streptococcal proteinase is probably the result of testing the enzyme at very low concentrations, dictated by its scarcity. A possible alternative explanation is that residual SOR protein may interfere with transduction because earlier studies from this laboratory (Hill and Wannamaker, 1968) suggested that the SOR protein may be relatively resistant to this enzyme. However, current studies in this laboratory have indicated that, at least in their extracted form, both M and SOR proteins are exquisitely sensitive to streptococcal proteinase as well as to trypsin and that the difference in sensitivity to streptococcal proteinase (less than 10-fold) is not consistent or impressive.

There was marked variability among group-G strains in the results obtained with trypsin treatment. Knowledge about the surface components of group-G strains is scant, but type-specific protein antigens functionally similar to M proteins have been identified in some strains (Lawal *et al.*, 1982), and indeed rare strains have been described with M protein that is serologically as well as functionally identical with that of group-A strains (Maxted and Potter, 1967). It is of interest that the opaque variant of one group-G strain resembled M+ group-A strains in its ability to multiply in normal human blood and in enhancement of transduction by trypsin treatment.

Maximal enhancement requires that trypsin be present during phage adsorption. Although we are

unable to explain this conclusively, one possibility is that receptors exposed by trypsin have a short half-life, in that they either decay or rapidly become inaccessible by the deposition of newly-synthesised proteins.

The findings of these studies raise important questions about streptococcal phage biology and provide a useful approach for enhancement of transduction of groups-A and -G streptococci.

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REFERENCES

- Cleary P P, Johnson Z 1977 Possible dual function of M protein: Resistance to bacteriophage A25 and resistance to phagocytosis by human leukocytes. *Infection and Immunity* **16**:280-292.
- Fischetti V A, Zabriskie J B 1968 Studies on streptococcal bacteriophages. II. Adsorption studies on group A and group C streptococcal bacteriophages. *Journal of Experimental Medicine* **127**:489-505.
- Fox E N, Krampitz L O 1956 Studies on the biosynthesis of the M-protein of group A hemolytic streptococci. *Journal of Bacteriology* **71**:454-461.
- Fraser C A M, Ball L C, Maxted W R, Parker M T 1979 Streptococcal skin sepsis among meat handlers. In: Parker M T (ed) *Pathogenic streptococci*. Reedbooks Ltd, Chertsey. pp 115-116.
- Friend P L, Slade H D 1966 Characteristics of group A streptococcal bacteriophages. *Journal of Bacteriology* **92**:148-154.
- Goeder H, Maxted W R 1961 External factors influencing structure and activities of *Streptococcus pyogenes*. In: Meynell G G, Goeder H (eds) *Microbial reaction to environment* (Society for General Microbiology Symposium no. 11), Cambridge University Press, pp 151-173.
- Griffith F 1934 The serological classification of *Streptococcus pyogenes*. *Journal of Hygiene* **34**:542-584.
- Hill M J, Wannamaker L W 1968 The serum opacity reaction of *Streptococcus pyogenes*: general properties of the streptococcal factor and of the reaction in aged serum. *Journal of Hygiene* **66**:37-47.
- Islam A K M S 1977 Rapid recognition of group-B streptococci. *Lancet* **1**, 256-257.
- Keil B 1971 Trypsin. In: Boyer P D (ed) *The Enzymes*, vol III, 3rd edn. Academic Press, New York, pp 249-275.
- Krause R M 1957 Studies on bacteriophages of hemolytic streptococci. I. Factors influencing the interaction of phage and susceptible host cell. *Journal of Experimental Medicine* **106**:365-384.
- Lawal S F, Coker A O, Solanke E O, Ogunbi O 1982 Serotypes among Lancefield-group G streptococci isolated in Nigeria. *Journal of Medical Microbiology* **15**:123-125.
- Maxted W R 1955 The influence of bacteriophage on *Streptococcus pyogenes*. *Journal of General Microbiology* **12**:484-495.
- Maxted W R 1957 The active agent in nascent phage lysis of streptococci. *Journal of General Microbiology* **16**:584-595.
- Maxted W R, Potter E V 1967 The presence of type 12 M-protein antigen in group G streptococci. *Journal of General Microbiology* **49**:119-125.
- McCarty M 1966 The nature of the opaque colony variation in group A streptococci. *Journal of Hygiene* **64**:185-190.
- Skjold S A, Maxted W R, Wannamaker L W 1982 Transduction of the genetic determinant for streptolysin S in group A streptococci. *Infection and Immunity* **38**:183-188.
- Skjold S A, Wannamaker L W 1976 Method for phage typing group A type 49 streptococci. *Journal of Clinical Microbiology* **4**:232-238.
- Slack M P E, Saunders F C, Mayon-White R T 1982 Streptococcal skin infections in meat workers in Oxfordshire. In: Holm S E, Christensen P (eds) *Basic concepts of streptococci and streptococcal disease*, Reedbooks Ltd, Chertsey, pp 28-29.
- Swanson J, McCarty M 1969 Electron microscopic studies on opaque colony variants of group A streptococci. *Journal of Bacteriology* **100**:505-511.
- Wannamaker L W, Almquist S, Skjold S 1973 Intergroup phage reactions and transduction between group C and group A streptococci. *Journal of Experimental Medicine* **137**:1338-1353.
- Wannamaker L W 1982 Phages, transduction, and phage-associated enzymes of group A, C, and G streptococci. In: Schlessinger D (ed) *Microbiology 1982*, American Society for Microbiology, Washington D.C., pp 112-116.
- Widdowson J P, Maxted W R, Grant D L 1970 The production of opacity in serum by group A streptococci and its relationship with the presence of M antigen. *Journal of General Microbiology* **61**:343-353.
- Williams R E O 1958 Laboratory diagnosis of streptococcal infections. *Bulletin of the World Health Organization* **19**:153-176.
- Wilson A T 1959 The relative importance of the capsule and the M-antigen in determining colony form of group A streptococci. *Journal of Experimental Medicine* **109**:257-270.