JOURNAL OF BACTERIOLOGY, Oct. 1969, p. 354-357
Vol. 100, No. 1

Distribution and Serological Specificity of Sialidase Produced by Various Groups of Streptococci

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Received for publication 16 June 1969

The occurrence of a streptococcal sialidase (designated St-sialidase) in culture fluids of various streptococci was investigated. St-sialidase was found to occur in strains belonging to groups A, B, C, E, G, H, and L, and the unclassified strains, *Streptococcus sanguis* and *Streptococcus uberis*. St-sialidase of group A was confined predominantly to types 4 and 22. St-sialidases, extracted from the culture fluids of some selected strains, were antigenic, eliciting the formation of antibody which effectively neutralized the enzymatic activity of the enzyme. Antisera to the St-sialidases of groups A, B, C, E, G, and L, and *Streptococcus sanguis* were produced in rabbits. The St-sialidases of groups A, B, and E streptococci were serologically distinct and group-specific. The St-sialidases from groups C, G, and L were serologically homologous, but distinct from St-sialidases of the other groups. Antiserum to the enzyme of strain 10557 (*S. sanguis*) cross-reacted with the St-sialidase of strain 9927 (*S. uberis*).

In the course of an investigation on streptococcal sialidase we have found two types of enzymes. The one was produced by a group K strain 6646 (K-sialidase; 3), and the other was produced by a variety of streptococci and termed St-sialidase (5). These two enzymes are distinct, and the difference between them was shown in the preceding paper (5). The present study deals with the screening of St-sialidase-producing strains among various groups of streptococci, and the serological relationship of these enzymes.

MATERIALS AND METHODS

Strains. The strains used in this study were obtained from the Type Collection Laboratory of The Institute for Medical Science (Tokyo University, Tokyo, Japan), Kanagawa Institute of Public Health (Kanagawa, Japan), T. Iimura (Toshima Hospital, Tokyo, Japan), and H. D. Slade (Department of Microbiology, Northwestern University Medical School, Chicago, Ill.).

Screening method of the St-sialidase-producing strains. Strains to be tested were cultured in the modified Todd-Hewitt medium as described in a previous paper (3). After incubating the cultures for 18 hr at 37 C, the culture fluids were separated from the cells by centrifugation at 5,000 × *g* for 30 min; 0.1 ml of each supernatant fraction was used for the determination of St-sialidase.

Assay procedure. The level of St-sialidase activity was determined as described in the preceding paper (5). The culture fluid containing the enzyme adjusted to 0.5 ml with 0.1 M acetate buffer (*p*H 5.5) supplemented with 300 μg of sialomucoid (BSM-St) and 0.01 M of $CaCl_2$ or $CoCl_2$. In the experiment dealing with the St-sialidase of group A streptococci $CoCl_2$ was used for the reason explained in the preceding paper (5). An incubation time of 30 min for the assay was used throughout these studies, except that 3 hr was used in the screening experiment for St-sialidase-producing strains of group A streptococci, in which the production of St-sialidase was relatively poor. The amount of sialic acid released was determined by the method of Aminoff (1), taking *N*-acetylneuraminic acid as standard. The definition of enzyme activity, in terms of unit, was described in the preceding paper (5).

Preparation of St-sialidase and substrate. St-sialidase preparations were made by successive fractionation with ammonium sulfate as described in the preceding paper (5). Fractions precipitated between 30 and 60% $(NH_4)_2SO_4$ saturation were the richest in all cases. The precipitate was dissolved in distilled water, dialyzed against distilled water for 4 days at 4 C, and lyophilized. The substrate (sialomucoid, BSM-St), which is specific for St-sialidase, was made from bovine submaxillary gland by the method described in the preceding paper (5).

Preparation of antisera. Antisera to various St-sialidases were made in rabbits by the injection of a mixture of equal volumes of St-sialidase in saline and complete Freund's adjuvant (Iatron Laboratories, Tokyo, Japan). Of the emulsion 2 ml containing 30 mg

of enzyme was injected subcutaneously to rabbits every other week for 3 or 4 months. Rabbit serum was tested for the absence of antibody against St-sialidase prior to the injection of antigen. So far we have succeeded in preparing antibodies against St-sialidases of strains T-4 62-2 (group A), 9925 (group B), SS188 (group C), SS193LI (group E), SS13 (group G), D167A (group L), and 10557 (*S. sanguis*).

Titration of St-sialidase antiserum. The level of antibody was determined by calculating the 50% inhibition titer. The titer is the reciprocal of the dilution of serum which neutralizes the activity of 50% of 0.07 unit of enzyme. Serum to be tested was inactivated by incubating in a water bath at 56 C for 30 min. Serial dilutions of serum in normal saline, starting from 1:4, were prepared in volumes of 0.1 ml and placed in test tubes. A 0.1 ml amount of enzyme (0.07 unit) in saline and 0.1 ml of 3% egg albumin were added to each tube. The assay tubes were incubated in a water bath for 1 hr at 37 C and stored overnight in the cold room at 5 C. On the next day the activity of the enzyme in each tube was determined, and the 50% inhibition titer was calculated as described earlier (4).

TABLE 1. *Survey of streptococci for the production of St-sialidase*[a]

Group and type	No. of strains tested	No. of strains Range of amount of sialic acid released (μg)				
		>20	20–10	10–5	5–1	<1
Group A[b]						
type 1	2				1	1
3	2				1	1
4	8	1	4	1	1	1
6	6				3	3
11	1				1	
12	4				2	2
22	7	5	2			
other types[c]	27					27
Group B	9	1	3	1	3	1
Group C	8	5				3
Group D	9				3	3
Group E	3	1			1	1
Group F	2				1	1
Group G	4	3				1
Group H	5	1			2	2
Group K	2				1	1
Group L	3	1	1			1
Group M	2				2	
Group N	1				1	
Group O	1				1	
Streptococcus viridans	4					4
S. salivalius	1					1
S. thermophilus	1					1
S. sanguis	1	1				
S. uberis	1	1				

[a] The production of St-sialidase of each strain is represented by the amount of free sialic acid released in the assay mixture containing 0.1 ml of each culture fluid. Values are number of strains for each range of amount of sialic acid released. Ranges of amounts are in micrograms.

[b] In the case of group A strains, the incubation time for the development of enzymatic activity was 3 hr, and for the other groups 30 min.

[c] Group A streptococci of the other types are as follows: type 5, 8, 9, 10, 13, 14, 15, 17, 18, 19, 23, 24, 25, 26, 27, 30, 31, 32, 33, 35, 39, 40, 41, 42, 43, 44, and 46.

RESULTS

Distribution of St-sialidase-producing strains. Table 1 reveals that the frequency of occurrence of St-sialidase-producing strains differs markedly between serological groups. Groups B, C, and G were rich in St-sialidase producing strains, and the remainder were poor. Among the group A streptococci, the positive strains were predominantly confined to types 4 and 22. Enzyme from the following strains was prepared for the serological study: 1232 (group A, type 4, 0.27 unit/mg), 1112 (group A, type 6, 0.005 unit/mg), 1229 (group A, type 22, 0.29 unit/mg), Y059H (group A, untypable, 0.43 unit/mg), 9925 (group B, 0.51 unit/mg), 8059 (group B, 0.55 unit/mg), 9926 (group C, 2.89 units/mg), SS193LI (group E, 1.38 units/mg), D166B (group G, 1.72 units/mg), F90A (group H, 0.021 unit/mg), and 9927 (*S. uberis*, 0.06 unit/mg).

Effect of egg albumin. Some enzyme prepara-

TABLE 2. *Effect of distilled water, normal saline, and egg albumin solution on the activity of St-sialidase in storage*[a]

Enzyme	Storage medium	Specific activity of St-sialidase (%)	
		After 1 day	After 7 days
1232	Distilled water	79.6	63.8
	Normal saline	79.0	59.7
	Egg albumin in distilled water (1%)	94.0	96.8
	Egg albumin in normal saline (1%)	88.7	98.5
H36B	Distilled water	37.4	30.3
	Normal saline	44.3	25.8
	Egg albumin in distilled water (1%)	107.5	159.5
	Egg albumin in normal saline (1%)	100.7	141.5
9926	Distilled water	86.7	95.3
	Normal saline	96.0	98.5
	Egg albumin in distilled water (1%)	92.3	91.8
	Egg albumin in normal saline (1%)	100.0	100.0

[a] A portion of each enzyme solution was drawn 1 and 7 days after storage. The specific activity of enzyme was determined, and represented in per cent, taking the original potency of each enzyme as 100.

TABLE 3. *Reactions, in terms of 50% inhibition titers[a], of antisera to St-sialidases of various groups streptococci with St-sialidases of homologous and heterologous groups streptococci*

St-sialidase of streptococcal strain	Antiserum to St-sialidase of streptococcal strain						
	T-4 62-2 (group A)	9925 (group B)	SS188 (group C)	SS193LI (group E)	SS13 (group G)	D167A (group L)	10557 (*S. sanguis*)
Group A							
T-4 62-2	1480.0	26.2	<4.0	10.3	4.0	<4.0	<4.0
1232	1780.0	24.1	<4.0	14.0	<4.0	<4.0	<4.0
1229	1450.0	35.1	<4.0	8.4	<4.0	<4.0	<4.0
Y020H	1460.0						
Group B							
H36B	<4.0	547.0	<4.0	7.0	<4.0	<4.0	<4.0
9925	<4.0	598.0	<4.0	<4.0	<4.0	<4.0	<4.0
8059		1650.0					
Group C							
SS188	8.5	28.0	25.8	8.9	212.0	59.5	9.6
9926	10.6	18.4	22.4	13.0	207.0	61.0	5.9
Group E							
SS193LI	<4.0	<4.0	<4.0	159.0	<4.0	<4.0	<4.0
Group G							
SS13	7.5	18.9	24.7	15.5	220.0	42.2	<4.0
D166B	9.3	19.8	71.7	23.1	319.0	94.5	9.8
Group L							
D167A	7.8	15.1	16.0	16.0	119.5	197.0	<4.0
Streptococcus sanguis	<4.0	<4.0	<4.0	4.5	<4.0	<4.0	38.2
S. uberis	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	25.8

[a] The 50% inhibition titer is the reciprocal of the dilution of serum which neutralizes the activity of 50% of 0.07 unit of enzyme.

tions lost activity during storage in solution. The stability of the St-sialidases in egg albumin was tested. St-sialidase was dissolved in distilled water, normal saline, or egg albumin solution (1%) and stored at 5 C, and then a portion of each solution was drawn at various time intervals for the determination of specific activity of the enzyme. Table 2 shows that the activity of St-sialidase was well preserved in the presence of egg albumin, and, moreover, the activity of H36B was apparently stimulated by egg albumin after 7 days of storage.

Serological study. Table 3 shows that antiserum to St-sialidase will neutralize the enzymatic activity of the enzyme from homologous group strains, and sometimes from heterologous groups strains. Antiserum to T-4 62-2 St-sialidase neutralized the enzymes of the group A strains, T-4 62-2, 1232, 1229, and Y020H, at almost the same levels. Antiserum to 9925 (group B) enzyme reacted strongly with the enzymes of the other group B strains. Antisera to the St-sialidases of group C, G, and L reacted with all St-sialidases of these groups. The St-sialidases of groups A, B, C, G, E, and L showed some cross-reactions against antisera to the St-sialidases of heterologous groups. Antiserum to the St-sialidase of strain 10557 (*S. sanguis*) inhibited markedly the St-sialidase of strain 9927 (*S. uberis*).

DISCUSSION

As demonstrated by results of the present study, the St-sialidases were produced by a wide variety of streptococci, particularly of the pathogenic streptococcal groups, but the frequency of occurrence of St-sialidase-producing strains differed markedly among the streptococcal groups. To obtain a satisfactory picture of the distribution of St-sialidase-producing strains among the streptococci, further studies should be done, because the number of strains used was restricted and the most efficient method of screening has not been obtained.

Serological study reveals that the St-sialidases of groups A, B, and E, were serologically distinct and group-specific, and the St-sialidases produced by strains of groups C, G, and L streptococci were serologically indistinct. The serological relationships among various St-sialidases resembled those of streptococcal hyaluronidase produced by groups A, B, C, and G streptococci (7). Hyaluronidase of group A streptococci was reported to be produced by strains of types 4 and 22 (2), and St-sialidase of group A streptococci was predominantly produced by the same types.

The St-sialidase of strain 9927 (*S. uberis*) reacted with antiserum to 10557 (*S. sanguis*) St-

sialidase at the same level as the homologous antigen, but not with the antiserum to SS193LI (group E) St-sialidase. *S. uberis* has been classified as a group E streptococcus (6). At present we have no data to explain this discrepancy.

ACKNOWLEDGMENT

We thank N. Shimizu, T. Iimura, T. Miyamoto, and H. D. Slade for kindly supplying the strains.

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