Growth Inhibition of Glucose-Grown Cariogenic and Other Streptococci by Saccharin *in vitro*

Harald A. B. Linke

Department of Microbiology, NYU Dental Center, New York

(Z. Naturforsch. **32 c**, 839–843 [1977]; received May 9, 1977)

Streptococcus mutans, Saccharin, Caries, Sweeteners, Streptococci

Sodium saccharin in concentrations from 0.02 to 20.00 mg/ml inhibits the *in vitro* growth of glucose-grown cariogenic and other streptococci, and suppresses, esp. at the higher concentration range, the fermentative acid production of these microorganisms to acid levels much lower than the critical pH of 5.5. These observations indicate a possible caries antagonism by saccharin.

Introduction

Recently, the *in vitro* physiological effects were studied of several sucrose substitutes and artificial sweeteners on growth pattern and acid production of 7 glucose-grown *Streptococcus mutans* strains [1], representing the 5 serological groups after Bratthall [2]. It was found that out of several sweeteners saccharin is the only compound which, depending on its concentration (0.02 to 20.00 mg/ml), significantly reduced or even inhibited the cellular growth of all tested *S. mutans* strains. Furthermore, in the presence of higher saccharin concentrations the final pH of the inoculated medium after 24 hours of incubation increased up to 1.8 units, which is far above the critical pH of 5.50 in dental plaque, necessary for determineralization of the teeth and caries formation [3, 4].

We now extended the aforementioned *in vitro* saccharin study to cover besides a greater variety of *S. mutans* strains, belonging to the 8 serotypes a to g and SL-1 after Bratthall [2] and Perch *et al.* [5], also other streptococci typical of viridans, enterococcus, pyogenic and lactic groups. Moreover, to acquire information on the inhibitory effect of saccharin on growth of streptococci in general, and to obtain proof that this growth inhibition is not limited to *S. mutans* but that also other oral *Streptococcus* species are affected by this compound.

Material and Methods

Bacterial strains

Twelve strains of *S. mutans*, representing the 8 serotypes a to g and SL-1 after Bratthall [2] and Perch *et al.* [5], were used in this study (Table I). In addition, fifteen *Streptococcus* species typical of viridans (2 *S. sanguis*, 3 *S. salivarius*, 3 *S. mitis*), enterococcus (2 *S. faecalis*, *S. faecalis var. liquefaciens*, *S. durans*), pyogenic (*S. pyogenes*, *S. agalactiae*) and lactic groups (*S. lactis*) were utilized (Table I).

Requests for reprints should be sent to Dr. Harald A. B. Linke, Department of Microbiology, NYU Dental Center, 421 First Avenue, *New York*, NY 10010, USA.

Table I. List of the 27 studied *Streptococcus* species.

Strain			Source
S. mutans	Serotype	Origin	
NCTC 10449	c	human	Dr. H. D. Slade,
AHT	a	human	Chicago, Illinois
B-13	d	human	USA
B-14	e	human	
FA-1	b	rat	
SL-1	SL-1/d/g	human	
OMZ-176	d	human	Dr. B. Guggenheim,
OMZ-175	f	human	Zürich, Switzerland
BHT	b	human	Dr. D. D. Zinner, Miami, Florida, USA
HS-6	a	hamster	Dr. R. J. Fitzgerald, Miami, Florida, USA
P-4	e	human	Dr. S. Edwardsson,
B-2	e	human	Malmö, Sweden
S. mitis ATCC 15909			American Type Culture Collection, Rockville, Maryland, USA
S. mitis ATCC 15910			
S. salivarius ATCC 9759			
S. salivarius ATCC 13419			
S. sanguis ATCC 10556			
S. sanguis ATCC 10557			
S. faecalis ATCC 19433			
S. faecalis var. liquefaciens			Midwest Culture Service, Terre Haute, Indiana, USA
S. pyogenes			
S. durans			
S. agalactiae			
S. lactis			
S. salivarius			
S. mitis			
S. faecalis			

Compounds

Sodium saccharin was obtained as a gift from the Sherwin Williams Co., Cleveland, Ohio.

Media

All *Streptococcus* strains were maintained in a complex medium composed of 2% glucose (Bacto-Dextrose, Difco) and 2% yeast extract (Difco) at pH 6.5 in screw cap test tubes containing 9 ml, sterilized in an autoclave for 10 min at 20 lb./sq. in. and 126 °C. The tubes were supplemented with 10% inoculum and were incubated at 28 °C. The cultures were transferred every 5 to 6 days.

Experimental approach

A stock solution of 2.8 g sodium saccharin/20 ml of distilled water was prepared and sterilized in an autoclave. The solution was then diluted 1 : 10, 1 : 100, and 1 : 1000 to yield the desired saccharin concentrations. For each test, 1.0 ml of the appropriate sweetener dilution and 0.5 ml of sterile water were added to 5.0 ml of a 2% glucose, 2% yeast extract medium at pH 6.5 in screw cap test tubes in duplicate. The final concentrations of sodium saccharin in the medium were 20.00, 2.00, 0.20, and 0.02 mg/ml. The tubes were then inoculated with 0.5 ml of a 48 hour culture of each of the 27 *Streptococcus* strains to yield a total volume of 7.0 ml. Inoculated tubes with 1.5 ml of sterile water added to the glucose-yeast extract medium served as a control. The test tubes were incubated in a gyrotory water bath shaker (New Brunswick Scientific, Model G 76) at 36 °C. After 24 hours the optical density, as a measure of growth, was determined at 546 nm using a Bausch & Lomb Spectronic 20 Spectrophotometer, and the final pH, as a measure of acid production, was read using a Fisher Accumet Model 230 pH/ion meter.

Results

I. *Effect of sodium saccharin on growth (OD) of Streptococcus species*

Sodium saccharin definitely exerts a growth inhibitory effect on glucose-grown *S. mutans* throughout the studied concentration range from 0.02 to 20.00 mg/ml; the magnitude of growth inhibition is basically proportional to the saccharin concentrations and affects most of the tested *S. mutans* strains, with the exception of the strains FA-1, SL-1, and B-2 where the two lower saccharin concentration (0.02 and 0.20 mg/ml) have little or no effect on

Table II. Growth (O.D. at 546 nm) of glucose-grown *Streptococcus* species in the presence of saccharin after 24 hours of incubation.

	Control (no additions)	Addition of sodium saccharin [mg/ml]			
		0.02	0.20	2.00	20.00
S. mutans					
NCTC 10449	0.68	0.66	0.60	0.30	0.11
AHT	0.81	0.73	0.65	0.48	0.09
B-13	1.25	1.10	1.05	0.92	0.37
B-14	0.91	0.84	0.85	0.56	0.08
FA-1	1.15	1.17	1.15	0.79	0.11
SL-1	0.64	0.65	0.62	0.44	0.27
OMZ-176	1.15	1.10	1.00	0.62	0.09
BHT	0.86	0.80	0.76	0.59	0.03
HS-6	0.84	0.62	0.60	0.49	0.07
P-4	0.79	0.75	0.72	0.38	0.11
B-2	0.60	0.59	0.59	0.39	0.09
OMZ-175	1.05	0.99	0.81	0.48	0.05
S. mitis ATCC 15910	0.69	0.72	0.69	0.55	0.11
S. salivarius ATCC 9759	0.95	0.98	0.97	0.68	0.09
S. salivarius ATCC 13419	0.83	0.80	0.82	0.51	0.15
S. sanguis ATCC 10556	0.53	0.50	0.43	0.23	0.03
S. sanguis ATCC 10557	0.71	0.71	0.67	0.40	0.05
S. faecalis ATCC 19433	0.61	0.60	0.61	0.54	0.22
S. faecalis	0.59	0.60	0.59	0.50	0.14
S. faecalis var. liquefaciens	0.52	0.58	0.54	0.41	0.20
S. pyogenes	0.37	0.29	0.29	0.18	0.01
S. durans	0.68	0.71	0.66	0.54	0.22
S. agalactiae	0.66	0.58	0.61	0.32	0.10
S. lactis	0.68	0.63	0.60	0.51	0.23
S. salivarius	0.66	0.66	0.62	0.52	0.15
S. mitis	0.56	0.54	0.51	0.31	0.02
S. mitis ATCC 15909	1.05	1.10	1.05	0.55	0.07

Table III. Acid production (pH) of glucose-grown *Streptococcus* species in the presence of saccharin after 24 hours of incubation.

	Control (no additions)	Addition of sodium saccharin [mg/ml]			
		0.02	0.20	2.00	20.00
S. mutans					
NCTC 10449	4.14	4.14	4.21	4.85	5.75
AHT	4.33	4.43	4.48	4.80	6.06
B-13	4.95	5.05	5.05	5.25	5.88
B-14	4.05	4.05	4.08	4.60	6.05
FA-1	4.39	4.35	4.36	4.73	5.98
SL-1	4.68	4.68	4.80	4.95	5.75
OMZ-176	3.80	3.80	3.95	4.45	5.75
OMZ-175	4.05	4.05	4.05	4.50	6.00
BHT	4.35	4.25	4.25	4.65	6.20
HS-6	4.38	4.46	4.45	4.80	6.10
P-4	4.19	4.25	4.25	4.80	5.92
B-2	4.44	4.45	4.45	4.82	6.03
S. mitis ATCC 15909	4.23	4.20	4.25	4.65	6.05
S. mitis ATCC 15910	4.40	4.33	4.34	4.34	5.53
S. salivarius ATCC 9759	4.26	4.23	4.25	4.60	5.77
S. salivarius ATCC 13419	4.35	4.33	4.34	4.76	5.56
S. sanguis ATCC 10556	4.55	4.55	4.59	5.04	6.21
S. sanguis ATCC 10557	4.48	4.46	4.48	4.68	5.98
S. faecalis ATCC 19433	4.45	4.45	4.48	4.73	5.37
S. faecalis	4.55	4.55	4.60	4.70	5.50
S. faecalis var. liquefaciens	4.50	4.50	4.45	4.78	5.40
S. pyogenes	5.15	5.05	5.12	5.35	6.45
S. durans	4.15	4.05	4.10	4.45	5.25
S. agalactiae	4.30	4.35	4.40	4.80	5.65
S. lactis	4.25	4.25	4.28	4.55	5.30
S. salivarius	4.55	4.52	4.60	4.75	5.45
S. mitis	4.55	4.50	4.50	4.95	6.35

growth (Table II). No relations could be found between serotypes and growth inhibitory effect of saccharin.

The other *Streptococcus* strains behave very similar, with the exception of the two species *S. faecalis*, *S. faecalis var. liquefaciens* and the oral streptococci *S. salivarius* and *S. mitis* (Table II). These four strains are also not or insignificantly affected by the lower saccharin concentrations. The two species *S. durans* and *S. agalactiae* seem to resist the lower saccharin concentrations but to a lesser degree than the four aforementioned species; these strains take a position in between these two extremes, no sensitivity or high sensitivity to the lower saccharin concentrations. All tested strains are highly affected by the 20.00 mg/ml saccharin concentrations; in some cases a total growth inhibition was observed.

II. *Effect of sodium saccharin on acid production (pH) of Streptococcus species*

The acid production of the glucose-grown *Streptococcus* species in the presence of sodium saccharin is summarized in Table III. The pH values of the controls indicate that all streptococci strains, with the exception of *S. pyogenes*, are high acid producers. The *S. mutans* strains produced exceptional low pH values during glucose fermentation; an extreme high acid producer is *S. mutans* OMZ-176, here a pH value of 3.80 was observed.

A significant decrease of acid production was obtained with an increasing saccharin concentration. In comparison to the control, the pH increased at the highest saccharin concentration (20.00 mg/ml) from 0.90 units (*S. faecalis var. liquefaciens*) up to 2.00 units (*S. mutans* B-14); the average pH increase for all *S. mutans* strains was found to be 1.65 units. The pH values at the highest saccharin concentration (Table III) range from 5.25 (*S. durans*) to 6.45 (*S. pyogenes*). These pH levels are in the average far above the critical pH of 5.50, necessary for demineralization of the teeth and caries formation. It seems likely that the increase in final pH in the presence of saccharin is reflected in the reduced growth.

Discussion

Dietary sucrose is utillized by oral streptococci to produce adhesive extracellular polymers (insoluble glucan), intracellular and extracellular storage polysaccharides (soluble glucan and fructan), organic acids (lactic acid) and serves as a carbon source for energy production (ATP) and growth. These aspects of dietary carbohydrate utilization, esp. by *Streptococcus mutans*, collectively contribute to their disease potential through their role in establishing dental plaque and in decalcification of the teeth. Since these nutritional aspects of the initiation of dental caries are well recognized [6], several possibilities can be offered to control this oral disease. The ban of sucrose from the list of human dietary products would be a desirable solution, although for several reasons not very practical; more useful single sucrose substitutes with a proven lesser cariogenic potential than sucrose would be glucose, fructose, mannitol, sorbitol, and xylitol [7, 8, 9]. Another more useful and feasible solution would be supplementing the sucrose diet with sucrose substitutes or artificial sweeteners for the purpose of "diluting" the sucrose intake; this would probably lead to development of dental caries at a rate significantly lower than a control population receiving solely a sucrose diet. Certainly, this would depend on the *in vivo* effects of this sweetener supplemented diet on the growth capability of streptococci inside the oral cavity. Therefore, it is of great interest to find out more about the possible synergistic effects of artificial sweeteners on the physiology of *S. mutans*, the etiological agent of dental caries, growing in a sucrose or glucose containing environment *in vitro*. Initially, a study was conducted on the physiological effects of sucrose substitutes and artificial sweeteners on growth pattern and acid production of glucose grown *S. mutans* strains *in vitro* [1]. A glucose system rather than a sucrose system was chosen to avoid excessive glucan formation and clumping of the cells. The results of this study suggested that sodium saccharin may have some potential in reducing the incidence of dental caries [10]. Therefore, the "saccharin effect" was investigated in more detail in this *in vitro* study, covering a larger number of *S. mutans* strains as well as a broader spectrum of *Streptococcus* species. The results indicate that saccharin at the appropriate concentration in general inhibits all tested *Streptococcus* species.

At this moment we do not know the mechanism of growth inhibition by saccharin; this compound could either interfere with the cell transport mechanism or with vital metabolic enzymes. Both the free saccharin as well as saccharin salts cause the same inhibitory effect on cellular growth.

Furthermore, the final pH of the medium as compared to the control increased at the highest saccharin concentration (20.00 mg/ml) up to 2.00 units, indicating that this amount of saccharin due to the reduced growth causes a reduction of the acid level of the medium to a pH which is far above the critical pH of 5.50, necessary *in vivo* for the initiation of demineralization of the oral hard tissues. These *in vitro* results indicate a possible caries antagonism by saccharin. Indeed, Grenby [7] found in a study involving 24 student volunteers receiving a diet supplemented with a low-calorie sweetener, composed of glucose with 0.4% sodium saccharin, that the amount of formed dental plaque, which was also higher in protein content, was significantly lower than in the control group. The average intake was 42 g sweetener or 168 mg sodium saccharin per day per test person for a 3-day test period.

At this point we also have to take into consideration, that due to FDA regulations, saccharin has been removed from the "generally-recognized-as-safe" list of food additives [11]. However, after the ban of cyclamate from the artificial sweetener market in 1969, at present, saccharin is still the commercially most important artificial sweetener in the U.S.A.

There is an urgent need to further test this inhibitory system *in vivo;* an animal study should be conducted with hamsters or other rodents on a cariogenic diet supplemented with saccharin. Such an experiment will aid answering the following key question: Would a population on a sucrose diet supplemented with saccharin for the purpose of diluting the sucrose intake develop caries at a rate significantly lower than a control population solely on a sucrose diet?

We wish to express our gratitude to Dr. Donald A. M. Mackay at Life Savers, Inc., New York, for a grant-in-aid in support of this study. The technical assistance of Byron Baker and Mariann Renna is greatfully appreciated, and we thank Sherry Johanas for preparation of the manuscript.

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