Cysteine Toxicity for Oral Streptococci and Effect of Branched-Chain Amino Acids

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Cysteine was bactericidal to strains of *Streptococcus mutans* and *S. salivarius* in concentrations that were nontoxic to *S. sanguis*, *S. milleri*, or *S. mitior* when these microorganisms were incubated in a saliva protein-based synthetic medium. Cysteine toxicity for *S. mutans* also occurred after incubation in synthetic base medium supplemented with amino acids as the nitrogen source for growth. The bactericidal effect of cysteine for *S. mutans* or *S. salivarius* in the saliva protein medium was influenced by the cysteine oxidative activity associated with the saliva protein fraction. Valine alone or in combination with leucine or isoleucine was effective in overcoming cysteine toxicity for susceptible strains of *S. mutans* or *S. salivarius*. Cysteine toxicity for these oral streptococci may be due to cysteine inhibition of an enzymatic step in the valine-leucine biosynthetic pathway.

Although cysteine has been reported to be toxic for a number of microorganisms and fungi (2, 3, 12, 13, 15, 16, 18, 23), different mechanisms of inhibition account for its toxicity. For example, cysteine toxicity for *Escherichia coli* has been shown to be due to a cysteine-specific inhibition of branched-chain amino acid synthesis (13, 15, 16). Conversely, a mechanism involving hydrogen peroxide generated during the aerobic oxidation of cysteine has been suggested to account for the toxicity of cysteine for *Pep-tostreptococcus anaerobius* (3) or *Salmonella typhimurium* (12).

Cysteine has been established to be among the amino acids which are essential to the growth of the oral group of streptococci (7-9, 21). We have previously demonstrated (5, 6) that whereas *Streptococcus mutans* and *Streptococcus sanguis* can metabolize the protein fraction of saliva as an in vitro amino nitrogen source for growth in a synthetic base medium test system, inclusion of small amounts of exogenous cysteine (50 µg/ml) in the medium results in some enhancement of microbial growth. Recently, in using this test system to compare the growth responses of *S. mutans* and *S. sanguis* on the protein fraction of saliva obtained from patients during and after radiation therapy for treatment of head or neck tumors, we encountered a situation in which none of the salivas examined supported the growth of *S. mutans*, but they did support at least some growth of *S. sanguis*. Further investigation revealed that the lack of growth of *S. mutans* on these salivas appeared to be caused by the cysteine in the synthetic base medium

rather than by growth-inhibitory properties directly associated with the salivas. Because of our interest in factors which may influence the growth of the oral streptococci, particularly on saliva proteins, this study was undertaken to further examine the potential toxicity of cysteine for *S. mutans* and other species of oral streptococci.

MATERIALS AND METHODS

Microorganisms. All strains of oral streptococci used in this study were obtained from the culture collection of the Miami Veterans Administration Medical Center Dental Research Unit. Stock cultures were maintained in Trypticase (BBL Microbiology Systems, Cockeysville, Md.) glucose broth (8) containing 0.5% calcium carbonate. They were transferred once weekly in this medium with incubation at 35°C for 18 to 20 h under a 90% N₂-10% CO₂ atmosphere.

Collection and treatment of saliva. Whole saliva was collected from a single donor source with salivary flow stimulated by chewing on sterile, washed rubber bands. The saliva was clarified by centrifugation (15,000 × g, 20 min), and the supernatant was concentrated to 5 ml by membrane ultrafiltration (5) to obtain a protein fraction of constituents >10,000 daltons. These saliva protein concentrates were placed in selective membrane dialysis sacks (3,500 dalton cutoff; Spectrum Medical Industries, Los Angeles, Calif.) and dialyzed overnight against 2 liters of distilled water at 4°C. The dialyzed preparations were divided into 1-ml portions and stored at -20°C until needed.

Description of test growth media. The control growth medium was a saliva protein-based medium (SPM) which consisted of the basal portion of the medium of Reiter and Oram (19) (synthetic base medium; SBM) and to which was added 380 µg of protein from the

dialyzed saliva protein concentrate per 400 μ l of medium as the nitrogen source for growth. The SBM contained glucose (1% wt/vol), mono- and dibasic potassium phosphate, purine and pyrimidine bases, minerals, vitamins, and 50 μ g of cysteine per ml. For the growth inhibitor tests, the cysteine content was adjusted to the desired level. SBM did not support the growth of any of the oral streptococcal strains used when the saliva protein fraction was omitted.

For some tests, amino acid supplements replaced the saliva protein fraction as the nitrogen source for growth in SBM. Amino acid supplement A contained (mg per 100 ml of medium): glutamic acid, 320; histidine, 7; methionine, 7; phenylalanine, 10; proline, 54; aspartic acid, 150; arginine, 45; lysine, 81; tryptophan, 10; and tyrosine, 4. The amino acid composition of supplement B was based on the minimal amino acid requirements for *S. mutans* (8) and contained methionine, aspartic acid, and lysine. The amino acids were dissolved in 50 ml of distilled water, and the pH was adjusted to pH 7.0. The amino acid solutions were filter sterilized by passage through 0.22- μ m pore size Millex (Millipore Corp., Bedford, Mass.) filters, and then portions were mixed with an equal volume of double-strength SBM to give the completed medium.

Growth inhibition tests. The oral streptococci were grown under a 90% N₂-10% CO₂ atmosphere in 10-ml amounts of Trypticase-glucose broth (8) to the mid-log phase of growth (ca. 6 h). Cells were collected by centrifugation, washed three times with 0.05 M potassium phosphate buffer, pH 7.0, and then resuspended in 10 ml of buffer. Stock solutions of cysteine or other sulfur-containing test agents were prepared fresh before use and filter sterilized. These agents singly or in combination were added to SPM, and then 400- μ l quantities of test media were inoculated with 5 μ l of washed-cell suspensions. In tests using SBM supplemented with amino acids, only the effect of cysteine was examined. The inoculated media were incubated for 40 min at 35°C under a 90% N₂-10% CO₂ atmosphere, after which 50 mg of glass beads (1.0 to 1.05 mm diameter) were added, and growth was dispersed by Vortex mixing (1 min). Serial dilutions of the growth cultures were pour plated with Trypticase-glucose agar, and the plates were incubated under a 70% N₂-10% CO₂-20% H₂ atmosphere (8) for 48 h at 35°C. The percent culture survival after the 40-min incubation period was calculated from $100 - [(NI \div NC) \times 100]$, where NI is the number of CFU per milliliter obtained in the medium containing inhibitor and NC is the number of CFU per milliliter obtained in the control medium (without inhibitor) after 40 min. Microscopic examination of dispersed growth cultures indicated that one CFU was derived from chains of four to eight cells.

Effect of branched-chain amino acids on cysteine inhibition. For tests on the effect of branched-chain amino acids on cysteine inhibition, SPM or the amino acid media contained 4.18 mM cysteine to which was added 0.5 mM leucine, 0.5 mM isoleucine, or 1.0 mM valine, singly or in various combinations. Growth inhibition assays in the presence or absence of branched-chain amino acids were performed as described above.

Sulphydryl (SH) determination. Before and after exposure to cysteine, growth cultures were centrifuged, and the cysteine content (as SH) of the cell-free

medium was determined as described previously by Janolino and Swaisgood (14). Briefly, 100 μ l of sample was transferred to a 5.0-ml volumetric flask and diluted to 1.0 ml with water. A reagent blank was prepared with water. Then, 3.0 ml of 0.067 M sodium phosphate buffer, pH 8.0, and 50 μ l of 0.01 M 5,5'-dithiobis(2-nitrobenzoic acid) (prepared fresh in buffer) were added in rapid succession, and the final volume was adjusted to 5.0 ml. After 20 min at ambient conditions, the color intensity was measured at 412 nm in a Gilford 240 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio), using a 1-cm light path quartz cuvette. After correction for background absorbance, the SH content was calculated from micromoles of SH per milliliter = $10 \times 0.367A_{412}$, where A_{412} is the absorbance at 412 nm.

Chemicals. Cysteine, other amino acids, glutathione (oxidized or reduced), dithiothreitol, and 5,5'-dithiobis(2-nitrobenzoic acid) were products of Sigma Chemical Co., St. Louis, Mo. All other chemicals used were of the highest quality commercially available.

RESULTS

Preliminary studies had indicated that whereas *S. mutans* VA-29R (type c) consistently produced seven to eight generations of growth in 6 h of incubation in SPM containing 0.84 mM cysteine, only two to three generations of growth were produced in medium which contained 1.67 mM cysteine. Inhibition of *S. mutans* by cysteine was also evident in studies in which the effect of cysteine concentration on survival of *S. mutans* VA-29R and *S. sanguis* in SPM or SBM supplemented with amino acids was compared. These results (Table 1) showed that whereas cysteine concentrations below 1.67 mM were not toxic to *S. mutans* in SPM, increasingly higher cysteine concentrations caused a proportionately greater reduction in culture survival. A similar toxic response to cysteine was also observed when the tests were conducted in the two defined amino acid test systems. Conversely, survival of *S. sanguis* 903-1600 in SPM was not affected by the cysteine concentration of the medium, and only a minor toxic response was observed in defined amino acid medium A. This organism was not tested in defined amino acid medium B because this medium was deficient in required amino acids.

The potential toxicity of cysteine toward other antigenic types of *S. mutans*, as well as other oral streptococcal species, was examined by using SPM containing 4.8 mM cysteine. In the case of *S. mutans*, survival of the test organisms was not affected after incubation in the control medium (without cysteine), but most strains showed at least some sensitivity to cysteine toxicity (Table 2); strains VA-29R (type c), B-13 (type d), LM-7 (type e), and QP50-1 (type f) were the most strongly affected. Under similar test conditions, 4.18 mM cysteine was also toxic to three of four strains of *Streptococcus salivar-*

TABLE 1. Comparison of effect of cysteine concentration on survival of *S. mutans* VA-29R and *S. sanguis* 903-1600 in SPM or in SBM containing amino acid supplements A or B as a nitrogen source for growth^a

Cysteine concn (mM)	% Survival after 40-min exposure ^b				
	<i>S. mutans</i>			<i>S. sanguis</i>	
	SPM	SBM + A	SBM + B	SPM	SBM + A
0.00	100.0	100.0	100.0	100.0	100.0
0.84	100.0	100.0	100.0	100.0	95.6 ± 6.0
1.67	70.1 ± 2.0	100.0	77.0 ± 4.0	100.0	91.6 ± 1.8
4.8	15.6 ± 3.0	27.8 ± 1.3	22.9 ± 2.0	100.0	93.2 ± 2.0
6.89	2.8 ± 0.3	10.7 ± 3.1	13.3 ± 3.0	100.0	99.7 ± 0.3

^a The nitrogen source of the SPM was 380 µg of dialyzed saliva protein fraction per 400 µl of medium. Initial cell populations were 7.7×10^6 CFU/ml for *S. mutans* and 1.2×10^7 CFU/ml for *S. sanguis*.

^b Data values are mean ± standard error for three independent experiments.

ius (decrease in survival ranged from 60 to 90%), but *S. sanguis* (6 strains), *Streptococcus milleri* (1 strain), and *Streptococcus mitior* were not inhibited by cysteine even at concentrations as high as 8.36 mM (data not shown).

The effects of various sulfur-containing agents on the survival of the indicator strain *S. mutans* VA-29R in SPM were tested to determine if the toxic effect was specifically due to cysteine. In these experiments, cystine, reduced or oxidized glutathione, dithiothreitol, and ferrous sulfate at concentrations of 1.0, 3.0, or 6.0 mM were nontoxic; and furthermore, when tested in combination with cysteine, none of them protected the organism from cysteine toxicity. The possibility that the toxic factor might be a specific oxidation product of cysteine was then examined by separately adding microbial cells or the saliva protein fraction to SBM containing different amounts of cysteine. After a 40-min incuba-

tion interval, the residual cysteine-SH content of the medium was determined by reaction with 5,5'-dithiobis(2-nitrobenzoic acid). The results of these tests (Table 3) revealed that only marginal and inconsistent oxidation of the available cysteine occurred in either the SBM control or in the medium to which microbial cells had been added. In contrast, addition of the saliva protein fractions resulted in substantial oxidation of cysteine in test systems containing 0.63 or 1.27 mM cysteine, but the percentage of total cysteine destroyed decreased at levels of 3.8 mM or greater.

Based on a comparison between these data and the survival data for *S. mutans* (Table 1), it appeared that a certain residual level of cysteine-SH was required for the toxic response. To confirm this possibility, we examined the effect of increasing the protein concentration in SPM at a constant level of cysteine (4.18 mM) on

TABLE 2. Survival of *S. mutans* serotypes in SPM in the presence or absence of 4.18 mM cysteine

Strain (type)	CFU/ml ($\times 10^7$) ^a			
	Initial	SPM only	SPM + cysteine	% Survival ^b
HS-6(a)	1.08 ± 0.4	1.86 ± 0.7	1.24 ± 0.5	66.7
AHT-12(a)	0.24 ± 0.03	0.72 ± 0.1	0.63 ± 0.2	87.5
BHT(b)	2.02 ± 0.02	2.10 ± 0.2	1.41 ± 0.2	67.1
FA1-R(b)	2.03 ± 0.8	2.25 ± 0.5	3.99 ± 0.9	100.0
VA-29R(c)	1.16 ± 0.2	1.79 ± 0.1	0.31 ± 0.1	17.3
GS-5(c)	1.33 ± 0.1	1.74 ± 0.3	0.83 ± 0.2	47.7
10449(c)	1.09 ± 0.2	1.25 ± 0.1	1.26 ± 0.4	100.0
OMZ-176(d)	0.70 ± 0.2	1.33 ± 0.5	1.02 ± 0.2	76.7
B-13(d)	1.28 ± 0.08	2.57 ± 0.01	0.47 ± 0.2	18.3
6715(d/g)	1.16 ± 0.4	1.67 ± 0.4	1.39 ± 0.07	83.2
K1-R(d/g)	1.40 ± 0.02	2.23 ± 0.5	1.56 ± 0.4	70.0
LM-7(e)	0.78 ± 0.02	1.09 ± 0.2	0.29 ± 0.03	26.6
B-2(e)	1.50 ± 0.1	2.42 ± 0.7	1.85 ± 0.4	76.4
QP50-1(f)	3.65 ± 0.4	4.02 ± 0.3	1.02 ± 0.4	25.4
OMZ-175(f)	1.19 ± 0.1	2.04 ± 0.5	1.34 ± 0.7	65.7
OMZ-65(g)	1.22 ± 0.1	1.55 ± 0.2	1.14 ± 0.08	73.5

^a Data values represent mean ± standard error of four independent experiments.

^b Survival is based on the difference in plate counts after 40 min of incubation in SPM (cysteine deleted) versus incubation in SPM containing 4.18 mM cysteine.

TABLE 3. Effect of saliva protein fraction or *S. mutans* VA-29R on oxidation of cysteine in SBM^a

Addition(s) to SBM	Min of incubation	$\mu\text{Mol SH/ml}$ determined at cysteine concn of: ^b			
		0.63 mM	1.27 mM	3.18 mM	6.36 mM
Water	0	0.68	1.50	3.49	5.22
	40	0.57 (16)	1.67 (0)	3.06 (12)	5.84 (0)
Cells	0	0.42	0.80	3.95	6.80
	40	0.36 (11)	0.58 (28)	3.50 (11)	7.20 (0)
Saliva	0	0.45	1.36	3.09	6.84
	40	0.15 (67)	0.47 (67)	3.08 (0.4)	6.24 (8)
Saliva + cells	0	0.50	1.23	3.67	7.10
	40	0.15 (70)	0.52 (66)	2.50 (32)	5.60 (21)

^a Reaction mixture was SBM containing either water, microbial cells (1.5×10^7 to 2.0×10^7 CFU/ml), or saliva protein fraction (380 μg of protein per 400 μl).

^b Tabulated values are means of three separate experiments with values in parentheses indicating the percentage of total cysteine-SH which was oxidized in 40 min.

cysteine oxidation and subsequent survival of *S. mutans*. Only a small percentage of the total cysteine was oxidized at protein concentrations of 0.19 or 0.38 mg/ml, but a greater amount of the cysteine was oxidized at higher protein levels (Fig. 1). Culture survival curves which were obtained after a 40- or 120-min exposure interval clearly indicated that cysteine toxicity was related to the residual cysteine-SH content of the medium. The higher percentage of survival during the 120-min exposure interval was attributed to growth of surviving cells.

The bactericidal effect of cysteine against *E. coli* has been shown to involve an inhibition of branched-chain amino acid biosynthesis (13, 15, 16). To determine whether a similar mechanism might explain the toxicity of cysteine for *S. mutans* or *S. salivarius*, the potential of the branched-chain amino acids to overcome the cysteine-induced inhibition was tested with strains which were highly sensitive to cysteine toxicity. *S. mutans* FA-1R, which was not sensitive to cysteine, was included for comparative purposes. None of the organisms exhibited the characteristic toxic response to cysteine when a combination of valine, isoleucine, and leucine was added, and most organisms also responded more favorably to combinations of valine and leucine or valine and isoleucine than they did to the individual amino acids (Table 4). The branched-chain amino acids as a combination also eliminated cysteine inhibition of *S. mutans* VA-29R in the defined amino acid systems (Table 5), except in the case of defined amino acid medium B containing 6.89 mM cysteine.

DISCUSSION

In the experiments described above, cysteine concentrations ≥ 1.67 mM were toxic to *S. mutans* regardless of the nitrogen source available

for growth. The bactericidal nature of this toxic effect was evidenced by decreased viability when the cells were plated on a nutritionally adequate recovery medium. Although most strains of *S. mutans* tested exhibited a sensitivity to cysteine during growth in SPM, the magnitude of the toxic response varied with the specif-

FIG. 1. Effect of protein concentration on cysteine oxidation and survival of *S. mutans* VA-29R in SPM after a 40- or 120-min exposure to 4.18 mM cysteine. Symbols: ●, micromoles of cysteine-SH oxidized; ○, percent culture survival after a 40-min exposure (initial cell population, $2.27 \pm 0.12 \times 10^7$ CFU/ml); □, percent culture survival after a 120-min exposure (initial cell population, $1.85 \pm 0.12 \times 10^7$ CFU/ml). Intervals are standard deviation of the mean for three separate experiments.

TABLE 4. Influence of branched-chain amino acids on survival of *S. mutans* or *S. salivarius* after a 40-min exposure to cysteine in SPM^a

Addition(s) to SPM	% Culture survival after exposure						
	<i>S. mutans</i> strain					<i>S. salivarius</i> strain	
	FA1-R	VA-29R	B-13	LM-7	OP50-1	3	SS-2
None	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Cysh	100.0	19.6	25.5	10.6	7.2	45.3	13.3
Cysh + val, leu, ile	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Cysh + val, leu	100.0	83.2	86.7	87.0	48.2	85.4	83.6
Cysh + val, ile	100.0	100.0	100.0	69.5	74.2	100.0	57.1
Cysh + val	100.0	100.0	86.7	44.3	28.9	99.2	56.1
Cysh + leu	100.0	50.7	82.3	44.1	15.9	80.7	60.0
Cysh + ile	100.0	54.0	81.3	33.9	28.9	86.9	34.0

^a SPM contained 380 µg of saliva protein fraction per 400 µl, 4.8 mM cysteine (Cysh), and either 0.5 mM leucine (leu), 0.5 mM isoleucine (ile), or 1.0 mM valine (val), or a combination of these. Values are means of three separate experiments.

ic strain and its antigenic type. The concentrations of cysteine which were inhibitory to *S. mutans* were much higher than those required to cause inhibition of *P. anaerobius* (3) or *S. typhimurium* (12), but they were comparable to those required to attain ≥70% inhibition of *E. coli* (15, 16). Whereas cysteine toxicity for some strains of *S. salivarius* was also demonstrated, this amino acid was nontoxic to *S. sanguis*, *S. milleri*, and *S. mitior* at the levels used in this study.

This previously unrecognized inhibitory effect of cysteine on the growth of the oral group of streptococci could be a cause of anomalous growth response behavior in test systems containing exogenously added cysteine. Whereas this problem could occur with both *S. mutans* or *S. salivarius*, it might be particularly troublesome with respect to the former species because different antigenic types, and even strains of the same antigenic type, display considerable variation in sensitivity to cysteine (see Table 2). Thus, any experimental test system which contains ≥1.67 mM cysteine should be considered potentially growth inhibitory for *S. mutans* or *S. salivarius* and should be pretested for cysteine toxicity for these organisms. It is possible to

alleviate this problem either by adding branched-chain amino acids to such test systems or by reducing the cysteine concentration.

The close similarity with respect to the effect of cysteine concentration on *S. mutans* in the SPM or defined amino acid test systems suggested that cysteine toxicity for this organism was not due to a specific interactive effect between cysteine and the saliva proteins or the other amino acids. We examined the possibility that the inhibitory effect was due to direct inhibition by cysteine itself or was caused by an oxidation product of cysteine. It is known that, under aerobic conditions, cysteine can undergo a metal ion-catalyzed auto-oxidation to cystine (20). Carlsson et al. (3) have reported that the hydrogen peroxide which is generated during this reaction is responsible for cysteine toxicity against *P. anaerobius*, and a similar mechanism has been suggested to account for the toxicity of cysteine against *S. typhimurium* (12). Although *S. mutans* is known to be sensitive to hydrogen peroxide (1), this process does not account for the observed effects of cysteine on the susceptible oral streptococci, since the exposure conditions used in this study (40 min, 90% N₂-10% CO₂ atmosphere) resulted in only minor auto-

TABLE 5. Effect of branched-chain amino acids (BCAA) on survival of *S. mutans* VA-29R in SBM containing amino acid supplements A or B after a 40-min exposure to different amounts of cysteine

Cysteine concn (mM)	% Culture survival ^a			
	(-)BCAA		(+)BCAA	
	SMB + A	SBM + B	SMB + A	SBM + B
0.0	100.0	100.0	100.0	100.0
0.84	100.0	100.0	100.0	100.0
1.67	100.0	83.6 ± 6.6	100.0	100.0
4.18	22.3 ± 4.3	27.3 ± 4.4	100.0	83.5 ± 11.6
6.89	6.9 ± 0.05	11.3 ± 2.0	91.3 ± 7.5	11.8 ± 0.35

^a Values are mean ± standard error for two separate experiments. (-)BCAA, Branched-chain amino acids not added to the test system; (+)BCAA, branched-chain amino acids present in the test system.

oxidation of cysteine, and because the greatest killing effect seemed to be associated with high residual levels of cysteine-SH. Further evidence in support of cysteine as the toxic factor came from the studies which indicated that cysteine, dithiothreitol, or reduced glutathione could not duplicate or overcome cysteine toxicity (data not shown) and from the studies which showed that conditions favoring cysteine oxidation by the saliva protein fraction coincided with greater survival of *S. mutans*.

The observation that the saliva protein fraction could cause substantial oxidation of cysteine was unexpected. Saliva has been reported to possess cysteine reductase activity (22), but to our knowledge, the existence of cysteine oxidative activity has not been previously demonstrated. This oxidative property was associated only with the protein fraction of saliva, was inactivated by heating (100°C, 5 min), and caused linear oxidation of cysteine in time-course studies (data not shown). After preparative electrofocusing of the protein fraction of saliva, the cysteine oxidative activity was present in a fraction of proteins consisting of five constituents having isoelectric points between pH 6.92 to 7.24. Although the saliva-associated, cysteine-oxidative activity may be attributable to an enzyme, possibly a sulfhydryl oxidase (14), the actual nature of this factor is presently under investigation.

The inhibition by cysteine of *S. mutans* and *S. salivarius* in SPM was completely overcome by a combination of valine, isoleucine, and leucine, and these amino acids also were effective in eliminating cysteine inhibition of *S. mutans* in the defined amino acid test systems. Branched-chain amino acids also have been shown to be effective in reversing cysteine toxicity for *E. coli* (13, 15, 16). Kari et al. (15) have suggested that the toxicity of cysteine for *E. coli* is due to the simultaneous inhibition by cysteine of homoserine dehydrogenase (10, 11) and acetohydroxyacid synthetase (17), which results in an auxotrophic requirement for threonine, isoleucine, valine, and leucine. On the other hand, Harris (13) has shown that isoleucine alone reverses the cysteine inhibition of *E. coli* and has concluded that the toxicity of cysteine is due principally to inhibition of threonine deaminase, a key enzyme of isoleucine biosynthesis. Since isoleucine alone was generally ineffective in eliminating cysteine toxicity for *S. mutans* or *S. salivarius*, it seems doubtful that cysteine inhibition occurs at the level of homoserine dehydrogenase or threonine deaminase. The greater relative effectiveness of valine, either alone or in combination with leucine or isoleucine, would indicate that the inhibition occurs in the valine-leucine biosynthetic pathway. Since the last three steps in

the synthesis of valine or isoleucine are catalyzed by the same enzymes (17), loss of the enzymatic function of any of these enzymes would result in an auxotrophic requirement for isoleucine as well. This mechanism of inhibition would explain the similarity in the effects of cysteine on *S. mutans* during growth on saliva proteins versus growth on amino acids. It could also explain why cysteine was not toxic to *S. sanguis*, *S. milleri*, and *S. mitior*, since these species generally require preformed branched-chain amino acids for growth (7, 9).

The requirement for exogenously supplied branched-chain amino acids to reverse cysteine toxicity for *S. mutans* in SPM was somewhat surprising since these amino acids were presumably available from the protein. We have previously demonstrated (4, 7) that both *S. mutans* and *S. sanguis* possess proteolytic activity toward salivary protein substrates and that this activity is related to the ability of these streptococci to metabolize saliva proteins as nitrogenous growth substrates (5, 6). Since the proteolytic activity associated with *S. mutans* is less hydrolytic and more specific than that of *S. sanguis* (7), one possibility is that the specific hydrolysis of saliva proteins by *S. mutans* does not result in the release of branched-chain amino acids in quantities sufficient to overcome the inhibitory effects of cysteine.

Although cysteine inhibition of *S. mutans*, and probably of *S. salivarius*, appears to involve an inhibition of branched-chain amino acid synthesis, the branched-chain amino acids did not completely eliminate the cysteine inhibition of *S. mutans* under certain growth conditions (see Table 5). Cysteine has also been shown to inhibit RNA synthesis (15, 18) as well as branched-chain amino acid synthesis in *E. coli* (13, 16), and it has been suggested these metabolic alterations may result in "unbalanced growth" of this organism (18). Furthermore, at cysteine concentrations above 2.0 mM, an additional secondary inhibitory effect of cysteine, involving an interaction with membrane-bound respiratory enzymes, may lead to energy depletion of the cells (15). Since cysteine could similarly affect the cell metabolism of *S. mutans* or *S. salivarius*, further studies are required to determine the specific sites of action of this amino acid in these oral streptococci.

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