Uptake and retention of tin by *S. mutans*

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Attramadal, A. & Svatun, B. Uptake and retention of tin by *S. mutans*. *Acta Odontol. Scand.* 1980, 38, 349 – 354

The uptake of tin by *S. mutans* from stannous fluoride or stannous chloride solutions was determined by atomic absorption spectroscopy. The uptake occurred rapidly, and the microorganism was shown to have a greater capacity and higher affinity to uptake of tin than of other metal ions tested. In 10 mM solutions, bound tin amounted to 17.5 per cent of the cellular dry mass.

The tin uptake was independent of cell metabolism. The cell bound tin could not be washed out with water or saline, but 84 per cent was removed by ethylenediaminetetraacetic acid solutions. When pH was lowered below 2, increasing loss of bound tin occurred.

It is suggested that the binding occurs to polyanionic structural polymers in the cell wall and the cell capsule.

Key-words: Oral bacteria; divalent cations

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In recent years, attention has been focused upon the use of chemical agents which exhibit an effect on dental plaque, either as an inhibitor of plaque formation, or as an inhibitor of plaque metabolism and acid formation. Both effects offer potential ways by which dental caries and periodontal disease may be reduced or prevented. Amongst the agents tested for inhibitory effect on dental plaque we also find fluorides, either in the form of the stannous or the sodium salt. Lilienthal found in 1956 (8) that stannous fluoride inhibited bacterial acid production *in vitro* more than sodium fluoride, and that this effect was not related to the fluoride content, but proportional to the tin concentration. Recent investigations in our clinic and laboratory have shown that stannous fluoride applied as mouthrinse or in toothpaste has a significant effect both as an inhibitor of acid formation in dental plaque (11), and also as an inhibitor of dental plaque formation (10, 12). Little of this effect could be attributed to the fluoride part of the compound, and it was further found that tin accumulated and was strongly bound in dental plaque (11), suggesting an uptake of tin by plaque bacteria.

Very few reports dealing with the binding and the effect of tin on microorganisms are found. The present study was undertaken to investigate the binding capacity and binding strength of tin in *Streptococcus mutans*, and the factors which influence this binding.

Received for publication, March 12, 1980

MATERIALS AND METHODS

Organism

Streptococcus mutans, strain OMZ 176, was used. Aerobic cultures were grown at 37° in Erlenmeyer flasks, in most of the experiments on tryptose phosphate broth (Difco Laboratories, Detroit, Michigan, USA). The bacteria were collected in either the exponential or stationary phase, usually after incubation for 24 hours, and then washed with distilled water at concentrations of 50–100 mg bacteria per ml.

Cation uptake

The uptake of tin was studied in bacteria samples of 100–200 mg, which were suspended in 70 ml aqueous solutions of the metal salt at concentrations varying between 0.005 mM and 10 mM. Stannous chloride was initially used as tin source, but as stannous fluoride proved to give a more stable solution, further experiments were performed with the fluoride salt. No difference was seen in the uptake of tin from the chloride and the fluoride salt. Except in experiments intended to determine the time effect of tin uptake, all test periods were 10 minutes. A series of experiments designed to observe the effect of temperature on the tin uptake was performed in thermo-controlled rooms at 37°, 22° and 4°. After the test period, the cells were collected by centrifugation at 2000 x g for 15 minutes, weighed and dried at 70° until constant weight.

The same procedure was employed to assess the binding to *S. mutans* of a series of other di- and tri-valent cations, as mentioned in Table II. The chloride salts were used, in the concentration range from 0.1 to 10 mM.

Tin retention

Bacteria which had taken up tin through exposure to 5 mM solutions of stannous fluoride for 10 minutes, were washed in five successive washings, each of 5 ml, using distilled water, saline or a 50 mM solution of the sodium salt of ethylenediaminetetraacetic acid (EDTA-Na). After this procedure, the tin content of the bacteria and the washing solutions was determined, and the percentage of tin washed out from the bacteria calculated.

The effect of pH on tin retention

The loss of tin at different pH levels was investigated by adjusting tin-containing bacteria suspensions to pH levels from 1 to 11, by the use of HCl, 0.1 mM acetate buffer, 0.1 mM HEPES buffer or NaOH. After 10 minutes, the samples were centrifuged, the tin content of bacteria and supernatant determined, and the tin loss from the bacteria calculated.

Competitive uptake between tin and other metal ions

A selection of the metals for which *S. mutans* had a high binding capacity was chosen for tests of the competitive uptake between tin and other metal ions. *S. mutans* samples were exposed to solutions containing varying combinations in concentrations of tin and one of the other metals, in the range between 0.1 and 10 mM. The test samples were centrifuged after 10 minutes, and the metal uptake by the bacteria calculated.

Metal analyses

The cells were digested in 1 ml suprapur concentrated nitric acid (E. Merck, Darmstadt, Germany). From the digests, samples for analysis were prepared by dilutions appropriate to the analysis range, in 0.2 per cent nitric acid. Atomic absorption spectroscopy analysis were performed with a Perkin-Elmer unit, model 360, equipped with a HGA-76 gra-

phite furnace (Perkin-Elmer, Norwalk, Connecticut, U.S.A.). Double distilled and deionized water was used, and all reagents, cells and water were analyzed for background contamination.

In calculating the metal uptake, the values were corrected for the metal content of the intracellular fluid, applying the theoretical value of 26 per cent for interspace in a system of close-packed spheres (4).

RESULTS

The uptake of tin by *S. mutans* occurred rapidly. With the technique used, the maximum uptake was completed within two minutes, which was the shortest time needed for separating the cells and the test solutions. Further exposure to the test solutions for up to 60 minutes gave no significant additional uptake.

At 10 minutes exposure time to test solutions containing stannous fluoride, *S. mutans* showed a notable ability to bind tin. The bacterial tin uptake in relation to the tin concentration in the test solutions showed a very avid binding up to tin concentrations around 0.1 mM in the ambient fluid. Above this level, the uptake occurred at a reduced rate. Within the upper limit of tin concentrations employed in the experiment (10 mM), the tin uptake did not, however, seem to reach any saturation level (Fig. 1). At 10 mM tin in the medium, cellular bound tin constituted 17.5 per cent of the dry cell mass.

S. mutans cells which had been exposed for 10 minutes to a solution containing 5 mM stannous fluoride showed little loss of tin when washed in distilled water or in saline, as can be seen from Table 1. The same procedure, using EDTA-Na solution, removed about 85 per cent of the bound tin. Analyses for fluoride revealed only traces left after the washings.

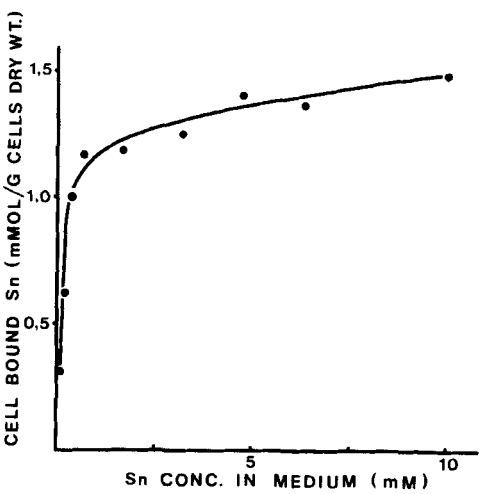

Fig. 1. The uptake of tin by *S. mutans* at varying tin concentrations in the medium.

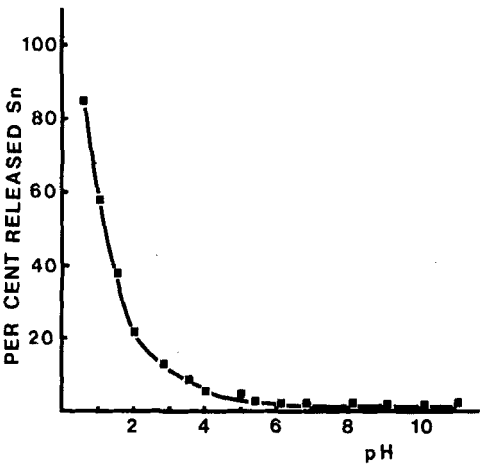

Fig. 2. The effect of pH on the loss of bound tin from *S. mutans*.

The hydrogen ion concentration had little influence on the tin retention at pH values above 3 (Fig. 2). Lowering the pH below 3 resulted in an increasing loss of bound tin, and at pH 0.5, 84 per cent of the initial bound tin was lost.

The uptake of various metal cations varied within a wide range, but the capacity of tin uptake surpassed by far that of any of the other cations tested (Table 2).

The bacterial uptake of tin was found to be the same at 37°, 22° and 4°. When the cell metabolism was arrested by treating the bacteria with 1 mM iodoacetate 10 minutes before and during exposure to tin solutions, the pattern of tin uptake by the bacteria was not affected.

No inhibition of the tin uptake was observed from the simultaneous presence of high concentrations of metals which showed relatively high uptake by the bacteria (manganese, copper, lead or aluminum). On the other hand, the presence of high concentrations of tin repressed the uptake of each of these metal ions. This is demonstrated in Table 3, which shows the binding of tin and copper at varied concentrations of each metal between 0.1 and 10 mM.

Table 1. *Release of tin from S. mutans by washings in five consecutive volumes of distilled water, saline or a 50 mM solution of the sodium salt of ethylenediaminetetraacetic acid. Data are averages of eight observations*

Solution	Percentage cell-bound tin removed by washing
Distilled water	10.7 ± 2.0
Saline	9.8 ± 3.4
EDTA	84.3 ± 4.9

DISCUSSION

Multivalent metal cations are normally accumulated in bacteria, either in the cell or in the cell wall as normal constituents (2, 3). At increasing metal concentrations in the medium, several investigations have shown cell membranes, cell walls and capsular material to bind large amounts of metal ions (5, 13). Tin has, however, rarely been included in such investigations (1). The present results show that *S. mutans* binds large amounts of tin, and it is also evident that the binding is very strong. Compared with the other metals tested in the present study, and compared with the reported uptake of metals by bacterial cells (1) and cell walls (2), the uptake of tin is unexpectedly high.

It is well known that stannous ions in aqueous solutions may form stannous hydroxide complexes, and that such complexes may be adsorbed onto solid-water interfaces. This hydrolysis occurs, however, slowly in stannous fluoride solutions at their native pH values around 3.5 (6), and there is thus little possibility that hydroxide complexes should develop to any significant degree during the 10 minute experimental period of the present study. The observed uptake of tin therefore conceivably occurs as a binding of stannous ions.

The readily uptake of tin at lower medium concentrations compared with the slighter uptake at higher concentrations indicate a difference in the cellular binding sites for tin. This difference may be due either to chemically different binding sites, or to a difference in the access to these sites. The replacement experiments indicate that tin and other metals at least to a certain extent have identical binding sites to the bacteria. They also show that the affinity for tin is larger than for other metals, as tin will displace other bound metals.

Several observations indicate that the bound tin is situated extramembraneously. This location is consistent with the rapid uptake, and also with the independence between tin uptake and cell metabolism. As EDTA is incapable of penetrating the bacterial cell membrane alone or in combination with metals, the results in Table 1 show that by far the major part of the bound tin is located outside the cell membrane.

The nature of the binding sites for tin is conceivably strongly acidic groups, according to the heavy loss of bound metal

Table 2. *The uptake of various metal ions at medium concentrations from 0.1 to 10 mM. Values are given as μmols metal/g bacteria dry weight, and are averages of three experiments*

Metal	Metal concentration in medium		
	10 mM	1 mM	0.1 mM
Sn	1432	978	328
Sr	3.5	0.4	0.006
Ni	90	12	2.4
Mn	197	18	5
Cu	133	49	18
Pb	437	237	83
Al	154	121	13
Zn	151	69	26

Table 3. *The simultaneous uptake of tin and copper for medium concentrations of the chloride salts between 10 and 0,1 mM. The cells were incubated for 10 minutes at 22°. The numbers, which are means of duplicate observations, express μmol metal/g bacteria dry weight*

		Sn in medium		
		0.1 mM	1 mM	10 mM
Cu in medium	0.1 mM	Cu 25 Sn 612	Cu 23 Sn 955	Cu 17 Sn 1658
	1 mM	Cu 92 Sn 674	Cu 24 Sn 1062	Cu 25 Sn 1617
	10 mM	Cu 241 Sn 667	Cu 134 Sn 1205	Cu 87 Sn 1562

when pH is shifted to values below 2. pK values in this range would point to phosphate groups. Such groups are abundant in structural polymers in the cell wall and the cell capsule, *e.g.* teichoic acids. These compounds are thus likely binding sites for metal cations (7).

The present study indicates that the accumulation of tin which is observed in dental plaque after oral rinses with stannous fluoride solutions is caused by a retention of the tin by plaque bacteria. However, recent experiments have shown that the plaque matrix also contains anionic groups (9), and it seems likely that these groups may also contribute to the total binding of stannous ions in dental plaque. As tin ions have inhibitory effect on bacterial metabolism and growth rate (1), the long-lasting retention of tin may cause a prolonged suppression of the bacterial metabolism. This effect obviously has interesting aspects related to preventive dentistry.

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