The amino acid composition of *Streptococcus mutans* and its culture medium supplemented with xylitol

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The amino and keto acid composition of the cells of *Streptococcus mutans*, strain Ingbritt, maintained and grown on a Trypticase-Phytone based medium without any added carbohydrate or supplemented with xylitol or glucose, was analyzed. The results showed no remarkable differences in the portions of individual amino acids liberated by acid hydrolysis from the cellular proteins of cells grown in the above mentioned media. However, the amount of free amino acids in the water extracts of the cells grown in the glucose medium differed considerably from those obtained from cells grown in the two other media. The amounts of free amino acids of the medium at the end of the growth period were higher in the glucose containing medium than in the two other media. The content of keto acids was lower in the cells grown in the presence of xylitol or without added carbohydrate when compared to those cells grown in glucose containing medium.

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Earlier studies dealing with the effect of xylitol on the growth and metabolism of *Streptococcus mutans*, strain Ingbritt (*Knuuttila & Mäkinen*, 1976, 1976), have shown that the maintenance of the cells of *S. mutans* for 18 months in a Trypticase-Phytone based medium, containing xylitol as the only added carbohydrate, did not give any indication of an adaptation to metabolize xylitol. However, slight but distinct growth was observed in the xylitol medium, which was accompanied by a simultaneous increase in extracellular proteolytic and intracellular aminopeptidase-like activity. Xylitol was not found to be responsible for these changes.

The aim of this study was to provide further information for an earlier assumption (*Knuuttila & Mäkinen*, 1975) that the cells grown without glucose might exploit energy and carbon sources other than carbohydrates. Therefore, we determined the total and soluble amino acid contents, and also the intracellular keto acids of *S. mutans* cells maintained and grown in the presence of glucose or xylitol, or without any added carbohydrate, as well as the soluble amino acid contents of the growth medium.

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MATERIALS AND METHODS

Cultivation of Streptococcus mutans

Streptococcus mutans (strain Ingbritt) was originally isolated from the human oral cavity and donated to this laboratory by Prof. Bo Krasse (University of Goethenburg, Sweden). A biochemical characterization of the test organism was carried out bimonthly as described by *Edwardsson* (1968). The cells were maintained and cultivated in Trypticase-Phytone based media as described by *Knuuttila & Mäkinen* (1975). The cultures of one liter were inbubated in 2 l Erlenmeyer flasks without aeration at 37°C. The turbidity was measured with a Klett-Summerson colorimeter (filter No. 62). The cells were maintained in three different stab cultures containing either glucose (0.25 g/100 ml) or xylitol (0.25 g/100 ml), or no added carbohydrate *(Knuuttila & Mäkinen,* 1975). The xylitol cells had been maintained for 30 months at +4 °C with monthly transfers to new medium.

Assays of amino acids

Amino acids in boiling-water extracts of *S. mutans* cells or in acid hydrolysates of cellular proteins were analyzed in the columns of a Perkin-Elmer KLA-5 automatic amino acid analyzer. The individual amino acids were separated, assayed with ninhydrin reagent, and recorded automatically. The quantity of each amino acid was determined by measuring peak areas. The amounts of different amino acids were expressed as relative portions of the total amino acid content in protein hydrolysates or in water extracts of free amino acid. The results presented in Tables I and II were confirmed with two or three separate analysis. The soluble amino acid contents of the media are expressed as relative changes between the values obtained before and after the cultivation.

The cells from three different stock cultures were prepared for amino acid analysis as follows. The cells grown in one liter of culture medium were separated by centrifuging for 10 min at 15,000 g (4 °C) with a Sorvall Superspeed centrifuge. Packed cells, washed once with cold water, were resuspended into cold distilled water. For extraction of the free amino acid pool, one 5 ml aliquot of aqueous cell suspension was kept for 10 min in a boiling water bath *(Raunio & Rosenqvist,* 1970), followed by centrifugation. The supernatant fluid was stored frozen at –20 °C before amino acid analysis. The other 5 ml aliquot of the same cell suspension was centrifuged to collect the cells for the assay of total amino acid content. The pellet, suspended in 3 ml of 6 N HCl, was hydrolyzed in a sealed test tube for 24 hours at 110 °C. The air-dried hydrolysate was washed three times with water and finally dissolved in 5 ml of distilled water. These samples were also stored frozen as above until analyzed. The samples for the analysis of the soluble amino acids of the media were taken simultaneously with other samples. The cells were removed and the samples were stored frozen before analysis. 1.6 ml of each sample without further treatment was used for amino acid analysis.

In order to make all three different cultures qualitatively and quantitatively comparable with each other, the cultivation was timed so that all cells were harvested at the end of the exponential growth phase which was reached in 6 to 8 hours. Furthermore, before dividing the suspensions of washed cells into 5 ml aliquots, the turbidities of the different preparations were adjusted to the same level with distilled water.

Keto acid assays

Keto acids were assayed principally with the diphenylhydrazin-method of *Katsuki et al.* (1971) using α-ketoglutaric acid as a standard. The samples were treated in the following way: 500 ml cultures of cells which had reached the end of the exponential growth

Table I. *Amino acid contents of* **Streptococcus mutans** *cellular proteins and free amino acid pool of the cells grown in a Trypticase-Phytone based medium supplemented with either glucose, xylitol or no added carbohydrate. Amino acid amounts are expresses as percentages of the total amino acid content of the preparation.*

Amino acid	Amino acids in cellular proteins			Amino acids in free amino acid pool		
	Glucose	Xylitol	No carbohydr.	Glucose	Xylitol	No carbohydr.
Tyrosine	3.29	3.65	1.81	1.60	2.15	2.85
Phenylalanine	4.23	4.87	2.80	4.40	4.30	2.85
Lysine	9.35	10.02	8.29	10.40	2.15	?
Histidine	5.37	2.59	2.01	2.80	3.20	2.85
Tryptophane	3.18	–	–	2.00	–	–
Arginine	2.97	3.88	2.80	4.00	1.07	2.85
Aspartic acid	7.29	8.12	7.34	8.00	16.12	17.01
Glutamic acid	10.60	11.80	11.48	12.40	26.88	28.57
Threonine	4.90	4.47	5.92	6.40	1.07	–
Serine	4.05	3.72	6.95	11.60	2.15	–
Alanine	16.10	15.25	18.27	17.60	26.88	25.71
Glycine	13.94	15.14	15.35	7.20	8.60	–
Methionine	2.01	2.30	4.18	2.40	1.07	–
Isoleucine	5.44	6.41	5.76	1.60	1.07	–
Leucine	7.74	7.78	7.02	7.60	3.24	–

Table II. *The relative changes (%) of soluble amino acids of media during the growth of the cells of* **Streptococcus mutans** *in a Trypticase-Phytone based medium supplemented with either glucose, xylitol or no added carbohydrate. The per cent changes are expressed per the same amount of cells in all three media.*

Amino acid	Glucose	Xylitol	No carbohydrate
Aspartic acid	+ 23	– 49	– 49
Threonine	+ 23	– 56	– 46
Alanine	+ 2	– 51	– 47
Glycine	0	– 35	– 44
Valine	– 7	– 45	– 34
Methionine	+ 2	– 62	– 37
Isoleucine	+ 4	– 58	– 36
Leucine	+ 5	– 47	– 36

phase, were centrifuged for 10 min at 15,000 g (4°C). The pellets were washed once with cold 0.9 % NaCl solution and suspended into 0.1 M Tris-HCl buffer (pH 7.0). The Klett-readings of the samples were adjusted to the same level with distilled water. Equal aliquots (3 ml) of these cell suspensions were supplemented with 6 mg of lysozyme, incubated for 60 min at 37°C, and sonicated three times (20 min each) with a MSE Ultrasonic Disintegrator (100 W Model, 9 mm probe). After centrifugation at 12,000 g for 10 min, the supernatant fluids were used for the keto acid assays.

The calculation of the dry weight of the cells was performed according to *Knuuttila & Mäkinen* (1975).

Chemicals

Glucose was a product of the British Drug Houses (Poole, Dorset, England), xylitol was obtained from the Finnish Sugar Company (Helsinki, Finland). α-Ketoglutaric acid was purchased from Sigma Chemical Company (St. Louis, U.S.A.). Unless otherwise mentioned, other chemicals were products of E. Merck (Darmstadt, Germany).

The water used was distilled and passed through an ion exchange column. The specific resistance of the water was approximately 1 MΩ-cm.

RESULTS

The results of the amino acis analyses of water extracts and HCl-hydrolysates of *S. mutans* cells grown in three different conditions are presented in Table I. There were no remarkable differences in the portions of individual amino acids of the cellular proteins between the cells of *S. mutans* grown either in the presence of glucose or xylitol, or in the absence of any added carbohydrate. Furthermore, the amino acid composition of the intracellular free amino acid pool of the glucose-grown cells was quite similar, both quantitatively and qualitatively, to the amino acid content of the three total protein hydrolysates. The only exceptions were the concentrations of serine, glycine and isoleucine.

On the other hand, the amounts of certain free amino acids in the water extracts of the cells growth in xylitol medium or in the medium with no added carbohydrate, showed considerable difference from the extracts obtained from the glucose-grown cells. The relative amounts of aspartate, glutamate, and alanine were increased significantly in the two former media, while the relative portions of lysine, arginine, threonine, serine, methionine, isoleucine, and leucine were decreased.

The relative changes of the soluble amino acid contents in three media during the growth of the cells are seen in Table II. The results showed a remarkable decrease in the amino acid content detected in xylitol and no carbohydrate medium. In glucose medium changes were very small except a slight increase in case of aspartate and threonine.

The results of the keto acid assays are illustrated in Fig. 1. The content of keto acids was remarkably lower in the cells grown in the presence of xylitol or without any added carbohydrate when compared to the cells grown in the presence of glucose. The differences were greatest at the end of the exponential growth phase, i.e. after approximately 12 hours of cultivation.

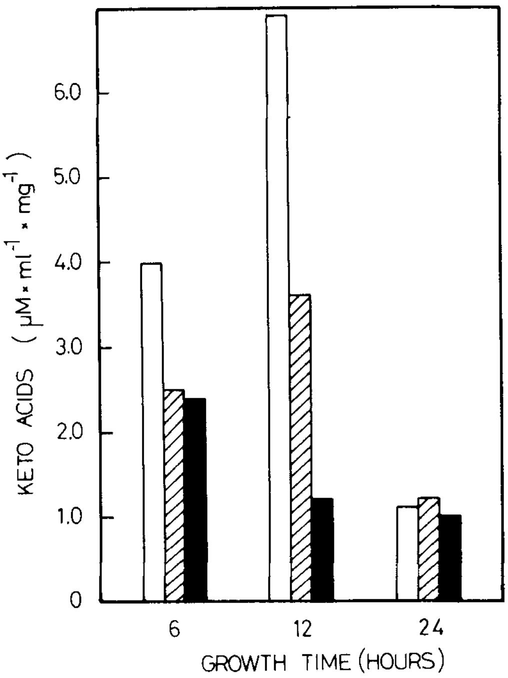

Fig. 1. Keto acid concentrations in *Streptococcus mutans* cells grown in Trypticase-Phytone-based medium supplemented with glucose (□), xylitol (▨) or no added carbohydrate (■).

DISCUSSION

In the present study, cells of *S. mutans*, strain Ingbritt, were grown in a Trypticase-Phytone based medium. The carbohydrate concentration of Trypticase-Phytone medium is small resulting in only a slight growth of the bacterial cells without any added carbohydrates. The complexity of this culture medium causes, however, difficulties in explaining the observed changes in the content and composition of the amino acid pools. Microbial pools, and particularly amino acid pools, are known to be extremely variable and markedly dependent on the nutritional complexity of the growth medium (*Holden*, 1962, *Cowman et al.* 1976).

Carlsson (1970) has shown that in addition to vitamin requirements, the aerobic growth of *S. mutans*, including strain Ingbritt, is dependent on one of the following amino acids: glutamine, glutamic acid, asparagine, or aspartic acid.

The results reported here indicate that the portions of aspartic acid, glutamic acid, and alanine in the free amino acid pool increased when the cells were grown in the presence of xylitol or in the absence of any added carbohydrate. *Krzeminski* (1975) has observed these amino acids to belong to the main part of the amino acid pool in cariogenic streptococci. This could be an indication of a more effective transport mechanism for these amino acids which are general amino group donors in transamination reactions and which, when converted into corresponding keto acids, are general intermediates of carbohydrate and energy metabolism as well as of biosynthetic pathways. The differences in pH of the media at the time the samples were taken can somewhat affect the transport of amino acids. The pH-values were 5.0 in glucose containing medium and 6.2–6.3 in the two other media.

The decreased amounts of lysine, arginine, threonine, serine, methionine, and leucine were found in the free amino acid pool obtained from the cells grown in the presence of xylitol. Wiht the exception of arginine, these amino acids were undetectable in the extracts from cells grown without added glucose. These five amino acids have been found to act as amino group donors in transamination reactions in most of the 27 different strains of *Streptococcus thermophilus* tested by *Nurmikko et al.* (1965). The lower keto acid concentrations found in the cells grown in the presence of xylitol or without added carbohydrate may not give support to the idea of increased transamination reactions of the above amino acids, which might lead to keto acid accumulation. It may, however, indicate a slowed penetration of these amino acids and/or their faster utilization. They may be metabolized effectively as soon as they have entered the cell from the extracellular amino acid pool or possibly been liberated from intracellular proteins by proteolytic enzymes. The above mentioned possibilities can explain also the changes in soluble amino acid content of the media.

The present result could thus be interpreted as a metabolic change toward the use of amino acids as carbon, energy, and nitrogen source in cells which have adapted to grow in the absence of fermentable carbohydrates. However, before reliable interpretations of the meaning and significance of the observed changes in the free, intracellular amino acid composition can be made, further studies are necessary. They should be conducted in chemically defined growth media in a chemostat, and in addition to the determinations of proteolytic activity and contents of free amino acids, the activities of various enzymes involved in amino acid metabolism should be assayed as well as the effect of various hydrolysis circumstances.

The present results are in accordance with those of a long-term feeding study in man. The consumption of a strict xylitol diet increased the nitrogen metabolism of dental plaque (*Mäkinen & Scheinin,* 1975; *Mäkinen, Lönnberg & Scheinin,* 1975). It was considered that the glucose-deprived bacteria of plaque obtained a part of their nitrogen and energy from peptides and proteins of the plaque extracellular phase and saliva.

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