

Basic amino acid in the pathogenesis of caries

Bazične amino kiseline slina u patogenezi karijesa

Ljubica Vranić
Paula Granić*
Zdravko Rajić**

Siget Health Center,
* Institute of Clinical
Laboratory Diagnostics,
Rebro Clinical Hospital
Center, Zagreb

** Unit of Children's and
Preventive Dentistry,
School of Dentistry,
University of Zagreb.

Summary

Amino acid pattern in total saliva was studied in 43 children with caries, aged 12-15 years, using the method of ion exchange chromatography. The results were compared to those obtained in a control group of 39 children without caries. In saliva from the children with caries, a significantly lower level of arginine (22.02 $\mu\text{mol/l}$), and a complete lack of histidine and its derivatives were observed when compared to the control group, where the concentrations of arginine and 1-methylhistidine were 28.36 and 26.34 $\mu\text{mol/l}$, respectively. The results obtained suggested that a decreased concentration of arginine, and a lack of histidine and its derivatives might imply an increased risk of caries.

Key words: *amino acids, caries*

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Introduction

Saliva as an immediate environment of the teeth is believed to play quite a significant role in the pathogenesis of caries. Caries is a function of two indivisible variables, enamel and nature of its environment. Dental enamel is an active surface, where physico-chemical processes of demineralization and remineralization proceed continuously depending on changes in salivary pH. On the one hand, the above mentioned processes can be designated as a cariogenic risk, and on the other, they appear to support the theory on tissue biologic adaptation in dependence of frequency and duration of exposure (1).

Only a few studies on the spectrum of free amino acids in saliva have appeared in literature to date, with different results reported. Initial studies were carried out by Kirch et al. (2), followed by Kesel et al. (3), and Battistone and Burnet (4). Jenkins (5) has identified 18 amino

acids, 9 of them regularly present, and the others found occasionally. Liappis and Hildebrand (6) determined free amino acids in saliva in relation to caries, but did not find any significant qualitative or quantitative differences in the concentrations of individual free amino acids. A study of qualitative content of free amino acids in saliva of children with caries and in saliva of children with phenylketonuria, however, in whom a low incidence of caries was observed, has revealed significant differences in the pattern of free amino acids (7).

Divergencies of the results obtained so far might be attributed to both the way of sampling and use of different methods of free amino acid determination in saliva. The present study was conducted under standardized and controlled conditions, employing a reliable method of identification and quantification of free amino acids in saliva of children with and without caries.

Material and methods

Subjects

Free amino acids were studied in saliva of 43 children with caries and 39 caries-free children aged 12–15 years. At this age, tooth exchange takes place, enamel maturation has not yet been completed and thus the teeth are susceptible to caries in a cariogenic environment.

Sampling

Native nonstimulated saliva of the subgingival region was examined. Salivary samples were collected between 7.30 and 9.30 a.m., after meal and oral hygiene. Study subjects were sitting with the head thrown backward, their mouth lightly open. The saliva collected from the oral cavity bed was transferred into sterile test tubes by an injection syringe and stored at -20°C until biochemical analysis, to inactivate bacteria and lysosomal enzymes degrading the saliva organic constituents. The time required for sampling was ≈ 10 min, and the amount of saliva 5–10 ml.

Method

Quantitative analysis of amino acids was carried out on a Beckmann 118 CL analyzer by ion exchange chromatography (Figure 1) (8). Salivary samples were first deproteinized with 50

mg of sulfosalicylic acid and centrifuged for 10 min at 3000 rpm. The supernatant was diluted with appropriate buffer and injected into the column. Three different buffers were employed: pH 2.83, pH 3.70 and pH 3.75, to separate acidic, neutral and basic amino acids. Then, identification and quantification of individual amino acids were performed.

For each group of subjects, the basic statistics was done on a Hewlett Packard 85 computer. Student's t-test was used to compare the mean values of particular parameters, and statistical significance calculated at the levels of $p < 0.05$ and $p < 0.001$.

Results

Results of the quantitative analysis of salivary amino acids in children with caries indicated the majority of amino acids belonged to the below listed ones, in order of decreasing concentrations: glutamine, proline, glycine and taurine (Table 1, Figure 2). Mean values between 20 and $32 \mu\text{mol/l}$ were recorded for alanine, serine, glutamic acid, arginine, tyrosine and phenylalanine. The greatest range of amino acid concentrations was observed for glutamine, proline and glycine, with highest values of 257 and $106.90 \mu\text{mol/l}$. Highest median values were found in the same amino acids, with the exception of

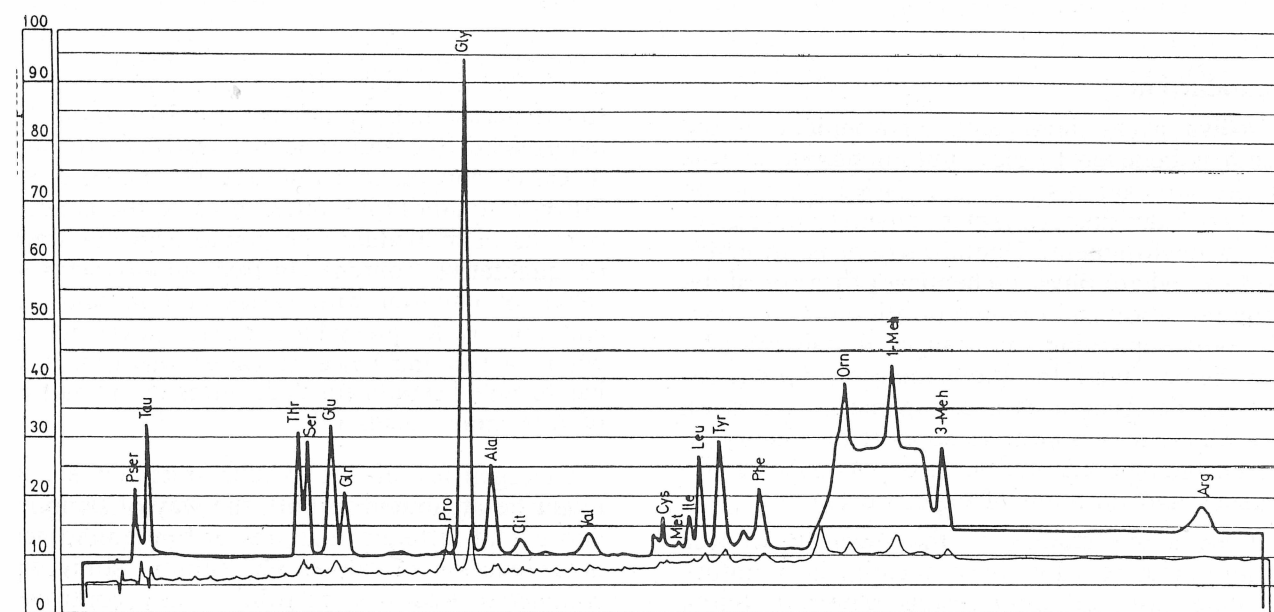


Figure 1. Chromatogram of Salivary Amino Acids
Slika 1. Kromatogram aminokiselina sline

Table 1. *Amino Acid Concentrations in the Children with Caries*
 Tablica 1. *Koncentracija aminokiselina u slini djece s karijesom* ($\mu\text{mol/l}$)

n = 43	\bar{x}	$\pm 1s$	Range		χ
			From	To	
Phosposerine	14,12	5,03	8,00	20,00	15,10
Taurine	50,22	21,97	29,00	85,00	50,00
Aspartic acid	15,56	20,62	0,50	50,30	9,30
Threonine	9,72	6,50	0,50	16,00	9,20
Serine	31,66	30,66	10,50	83,60	16,00
Glutamine	83,34	97,17	33,00	257,00	41,00
Proline	64,96	79,16	21,60	205,70	27,50
Glutamin acid	27,40	25,86	14,20	73,60	17,20
Citrulline	14,08	17,17	4,30	44,70	7,10
Glycine	61,06	27,28	35,70	106,90	51,70
Alanine	32,86	13,95	23,20	56,40	25,20
Valine	12,62	10,82	5,80	31,80	8,10
Isoleucine	7,22	11,42	0,50	27,40	2,90
Leucine	11,86	14,73	0,50	37,70	6,90
Tyrosine	20,44	14,62	12,00	46,50	15,00
Phenylalanine	20,30	21,80	8,10	59,20	11,60
Ornithine	9,34	2,53	6,60	12,10	8,60
Arginine	22,02	4,17	15,60	26,40	23,70

Table 2. *Amino Acid Concentrations in the Saliva of Caries-free Children*
 Tablica 2. *Koncentracija aminokiselina u slini djece bez karijesa* ($\mu\text{mol/l}$)

n = 39	\bar{x}	$\pm 1s$	Range		χ
			From	To	
Phosposerine	21,24	14,49	9,40	45,70	19,80
Taurine	54,96	29,53	36,50	105,00	42,70
Aspartic acid	5,30	6,57	0,50	12,30	0,50
Threonine	16,30	7,33	7,20	25,60	18,80
Serine	25,68	9,04	18,20	41,00	24,50
Glutamine	55,40	20,73	37,50	81,80	46,70
Proline	68,98	35,14	36,80	112,50	51,50
Glutamin acid	28,42	13,77	21,00	52,90	22,00
Citrulline	8,74	5,11	4,10	16,70	6,30
Glycine	89,80	44,97	45,90	151,30	65,50
Alanine	37,64	19,77	26,90	72,90	29,80
Valine	5,14	4,57	2,20	13,00	3,90
Isoleucine	4,54	2,63	0,50	7,50	5,0
Leucine	9,72	3,28	6,90	15,00	9,60
Tyrosine	19,54	11,35	11,90	38,40	12,90
Phenylalanine	11,58	0,82	10,80	12,60	11,30
Ornithine	23,52	23,03	5,40	61,80	15,30
1-Methylhistidine	26,34	10,64	17,00	44,40	23,10
Arginine	28,36	8,91	19,20	40,80	30,10

taurine, its median being 50 $\mu\text{mol/l}$, the same being also valid for the mean value of this amino acid.

In the saliva of caries-free children, the majority of amino acids in order of their concentrations belonged to glycine, proline, glutamine and taurine. Mean values between 21.00 and 47

$\mu\text{mol/l}$ were measured for phosphoserine, ornithine, serine, methylhistidine, arginine, glutamic acid and alanine. The greatest concentration range was recorded for glycine, proline, taurine and ornithine, with highest values of 151.20 and 105.10 $\mu\text{mol/l}$. These amino acids also had the highest median and mean values.

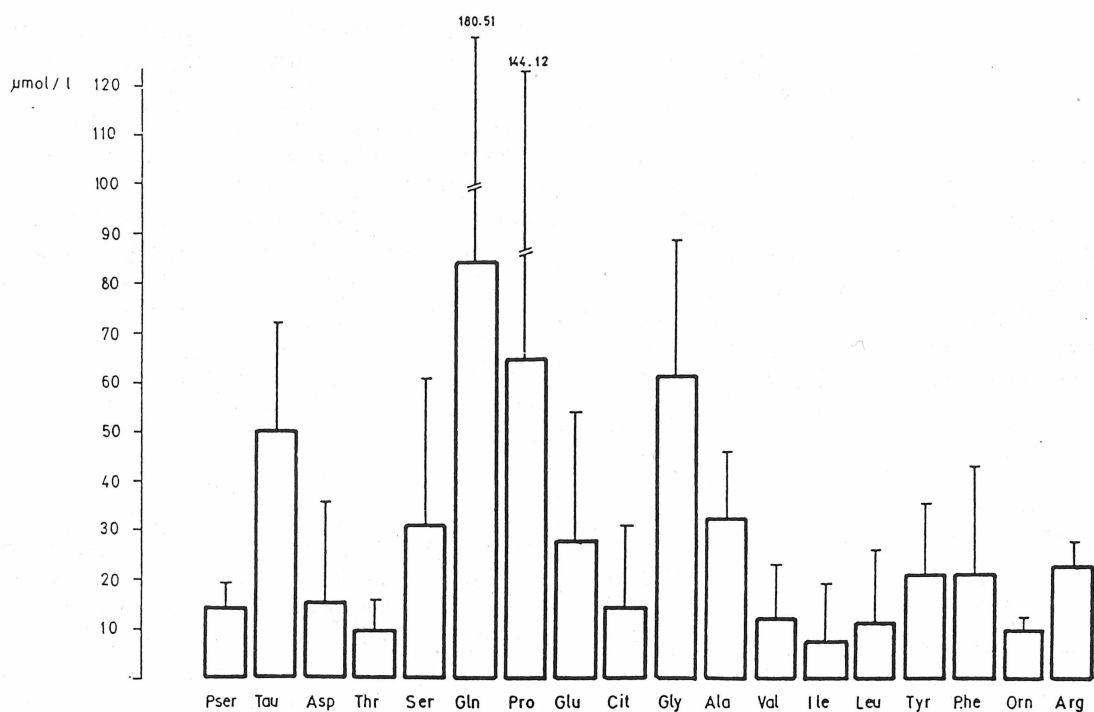


Figure 2. Amino Acid Concentrations in the Saliva of Children with Caries
Slika 2. Koncentracija aminokiselina u slini djece s karijesom

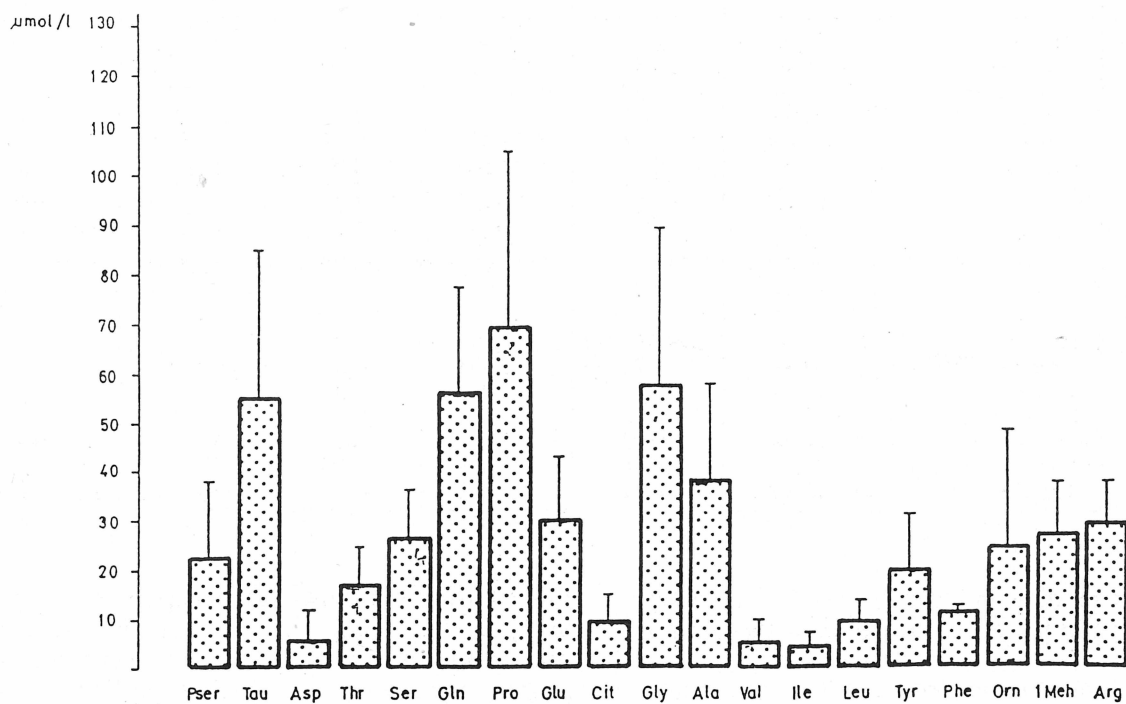


Figure 3. Amino Acid Concentrations in the Saliva of Caries-free Children
Slika 3. Koncentracija aminokiselina u slini djece bez karijesa

Discussion

Results of this study showed acetic and neutral amino acids to predominate in saliva of the children with caries, whereas in saliva of the caries-free children the acetic amino acids glutamine and aspartic acids were present in considerably lower concentrations. In saliva of the children with caries, higher concentrations of asparagine, glutamine, valine and phenylalanine, and lower concentrations of threonine, ornithine and arginine were found. An absolute lack of histidine and its derivatives was observed, which is in part consistent with the findings of Wandelt, who found increased amounts of aspartic and glutamic acids, suggesting that chelation, i.e. formation of metal complexes with amino acids, may have led to initial lesion and secondary to destruction of the organic matrix by the action of proteolytic bacteria (9).

In this study, no statistically significant differences were observed in the amount of amino acids secreted in the saliva of children with caries, as compared to the results obtained in the saliva of caries-free children ($p > 0.05$). This is consistent with the study of Liappis and Hildebrandt, who have not found any significant qualitative or quantitative differences in the concentrations of individual amino acids in a sample of 33 children with and 32 children without caries (6). The present study, however, revealed differences in the amino acid pattern. Thus, in the saliva of children without caries the concentration of arginine and its range were higher than in the group of children with caries, although the difference was not statistically significant. Another difference was brought about by the addition of the basic amino acid, 1-methylhisti-

dine, to the spectrum of free amino acids, observed in the saliva of healthy children, which did not occur in the saliva of children with caries. Arginine and histidine are conditionally considered essential amino acids in childhood, because of increased requirements or diminished synthesis needed for nitrogen balance maintenance. Therefore, their intake in nutrition is very important (10). Later in life, histidine also plays an important role in the composition of many proteins, due to its imidazole group contributing to the buffering capacity of plasma proteins and reaction at the catalytic sites of a number of enzymes.

Basic amino acids arginine and lysine improve calcium absorption by the formation of soluble complexes with calcium that maintain calcium in an absorbent form (5). This feature of basic amino acids might be important during the period of post-eruptive enamel maturation, which greatly depends on the immediate tooth environment, i.e. saliva. Immediately after eruption and during the first 14 days to 2 years following tooth eruption in particular, the final incorporation of minerals into the enamel occurs, the so-called enamel maturation. Pre-eruptive mineralization and post-eruptive maturation will determine the quality of dental tissues for the whole period of tooth life, i.e. susceptibility or resistance to caries and action of environmental factors.

The results obtained in this study of the salivary amino acid pattern in children with caries pointed to a conclusion that a decreased concentration of arginine and the lack of histidine could be considered an increased risk of caries.

BAZIČNE AMINO KISELINE U PATOGENEZI KARIJESA

Sažetak

Istraživan je aminokiselinski profil ukupne sline u 43 djece s karijesom u dobi od 12–15 godina metodom kromatografije na ionskom izmjenjivaču. Dobiveni rezultati uspoređeni su s rezultatima kontrolne skupine od 39 djece bez karijesa. U slini djece s karijesom nađeno je značajno manje arginina ($22.02 \mu\text{mol/l}$) i potpun nedostatak histidina i njegovih derivata u odnosu na kontrolnu skupinu, gdje je koncentracija arginina bila $28.36 \mu\text{mol/l}$, a 1-metilhistidin

Address for correspondence:
Adresa autora:

Ljubica Vranić
Siget health center
Aleja pomoraca bb
YU – 41000 Zagreb

u koncentraciji od 26.34 $\mu\text{mol/l}$. Na temelju dobivenih rezultata moglo bi se zaključiti da smanjena koncentracija arginina i nedostatak histidina, kao i njegovih derivata, ukazuje na povećani rizik prema karijesu.

Ključne riječi: *amino kiseline, karijes*

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