J Radioanal Nucl Chem (2009) 282:29–32
DOI 10.1007/s10967-009-0262-9

Elemental comparison in sound and carious human teeth by instrumental neutron activation analysis

M. Saiki · L. K. Adachi · E. M. Adachi

Received: 30 June 2009 / Published online: 28 July 2009

Abstract Instrumental neutron activation analysis (INAA) was applied to determine Ca, Cl, Mg, Mn, Na, Sr and Zn in human dentin and enamel tissues from carious and sound teeth. Comparisons were made between the results obtained in carious and sound dental tissues for dentin and enamel. The findings obtained were also compared with the published data. Accuracy and precision of the results were evaluated by analyzing certified reference material.

Keywords Trace element · Neutron activation analysis · Sound teeth · Carious teeth

Introduction

Very little is known about role of trace elements in teeth. The importance of trace elements in the prevention and in reduction of dental caries has not yet been established. The formation of dental caries is associated with infection by two groups of bacteria: *Streptococcus mutans* and *Lactobacilli*. However, there are indications that trace elements play an important role in caries resistance. For example, epidemiological studies have shown the role of fluorides in the reduction of dental caries by altering the solubility of dental tissue and transforming the size and shape of the crystallites [1, 2].

Caries remains one of the most common diseases throughout the world [3]. Today, people are living much longer so the study of factors that affect the incidence of dental caries are becoming a subject of great interest in order to preserve the natural teeth during the ageing process. Lately several investigators [1, 4–7] have analyzed human teeth to study the link between trace elements and dental caries.

The purpose of this study was to estimate and compare trace element concentrations in dentin and enamel tissues from human carious and sound teeth. While the trace element composition of whole teeth has been analyzed by several researchers [8–10] there are little data on the trace element composition of enamel separately. These determinations are important to increase the current knowledge of the elemental composition in each tissue of human teeth and the association of elements in the caries problems.

In this study instrumental neutron activation analysis (INAA) was applied to determine Ca, Cl, Mg, Mn, Na, Sr and Zn. INAA constitutes an advantageous method for dental tissue analysis as it enables simultaneous multi-element determinations and does not require sample dissolution.

Experimental

Samples

Samples of permanent human teeth were supplied by dental clinics. Eighteen dentin tissues being 9 carious and 9 sound plus also eighteen enamel tissues being 9 carious and 9 sound were analyzed. These teeth were extracted for

M. Saiki (✉) · E. M. Adachi
Instituto de Pesquisas Energéticas e Nucleares, IPEN-CNEN/SP, Av. Professor Lineu Prestes No. 2242, CEP 05508-000 São Paulo, SP, Brazil
e-mail: mitiko@ipen.br

L. K. Adachi
Faculdade de Odontologia, Universidade de São Paulo, Avenida Professor Lineu Prestes, No. 2227, CEP 05508-000 São Paulo, SP, Brazil

reasons of carious destruction or for clinical orthodontic reasons. The Ethics Committee, of the Instituto de Pesquisas Energéticas e Nucleares (IPEN—CNEN/SP) approved this research. The extracted teeth were washed thoroughly under tap water to remove saliva, blood and soft tissue of the gum. All the samples analyzed were from individuals living in the coastal city of Santos, Brazil. The dentist using appropriate tools separated the enamel from the dentin mechanically. All precautions were taken to avoid sample contamination.

For the analyses, each sample was ground to powder using an agate mortar. About 10–100 mg of each sample were weighed in clean polyethylene bags and heat-sealed. These bags were manufactured using polyethylene foils previously cleaned using diluted nitric acid solution and purified water.

Standards

Certified standard solutions of elements provided by Spex Certiprep, USA were utilized to prepare synthetic standards of elements. Aliquots of these solutions were pipetted onto small sheets of Whatman No. 40 filter paper, and after drying at room temperature, these sheets were folded and placed in clean polyethylene bags and also heat-sealed. Element Na was detected at very low concentrations in the filter paper and its contribution in the synthetic standards could be considered negligible. The amounts of the elements used for irradiation were (in μg): Ca = 1000.0; Cl = 500.0; Mg = 998.9; Mn = 10.04; Na = 100.0; Sr = 1001.7 and Zn = 36.0.

Neutron activation analysis procedure

Samples and synthetic standards of elements were irradiated at the IEA-R1 nuclear research reactor. Ten-second irradiations under thermal neutron flux of 6.6×10^{12} n cm^{-2} s^{-1} were used for Cl, Mg, Mn, Na and Sr determinations. Sixteen-hour irradiations under thermal neutron flux of 5×10^{12} n cm^{-2} s^{-1} were carried out for Ca, Na, Sr and Zn determinations. Elements such as Ba, Fe and Se were not determined due to the interference of a large bremsstrahlung background from $^{31}P(n,\gamma)^{32}P$ or due to their low concentrations in the samples. The radioisotopes ^{59}Fe and ^{75}Se could not be detected in the gamma spectra after about 2 months of decay time. After adequate decay times, the irradiated samples and standards were measured by a hyperpure Ge detector Model GC2018 from Canberra coupled to a DSA-1000 Multichannel Analyzer. The resolution (FWHM) of the system was 1.0 keV for 122 keV gamma-ray peak of ^{57}Co and 1.87 keV for 1332 keV gamma ray peak of ^{60}Co. Each sample and standards were measured at least twice for different decay times. Counting times from 200 to 50,000 s were used, depending on the half-lives or activities of the radionuclides considered. Spectra were collected and processed using Canberra Genie 2000 Version 3.1 spectroscopy software. The radionuclides measured were identified according to their half-lives and gamma-ray energies. The concentrations of elements were calculated by a comparative method. The area under peaks corresponding to the gamma-rays of ^{47}Ca at 159 and 1297.1 keV, ^{38}Cl at 1642.7 keV, ^{27}Mg at 843.7 and 1014.4 keV, ^{56}Mn at 846.7 and 1810.7 keV, ^{24}Na at 1368.6 keV, ^{85}Sr at 514.0 keV, ^{87m}Sr at 388.40 keV and ^{65}Zn at 1115.6 keV were used.

The quality of the analytical results was evaluated by analyzing the certified reference material (CRM), NIST 1486 Bone Meal provided by the National Institute of Standards and Technology (NIST), USA. This reference material was analyzed by applying the same experimental conditions used in the tooth analyses. The element concentrations of reference material were evaluated on a dry weight basis.

Results and discussion

Table 1 presents the results obtained in the analyses of certified reference material NIST 1486 Bone Meal together with its certified values [5]. The results obtained agreed with the certified values with relative errors lower than 6.0%. The results also presented good precision with relative standard deviations varying from 5.0 to 11.0%.

Results obtained in permanent dentins and enamels for carious and non carious teeth are presented in Tables 2 and 3, respectively. Comparisons were made between the results obtained for carious and non carious teeth by applying Student's *t*-test. These results presented in Table 2 indicate no significant difference between carious and sound dentins

Table 1 Concentration of elements in the certified reference material NIST 1486 Bone Meal

Elements	Mean ± SD	RSD, %	Er, %	Values of certificate [11]
Ca, %	27.60 ± 1.37	5.0	3.8	26.58 ± 0.24
Cl, mg kg^{-1}	352 ± 34	9.6	–	–
Mg, %	0.494 ± 0.041	8.3	6.0	0.466 ± 0.017
Mn, mg kg^{-1}	1.08 ± 0.12	11.1	–	(1)[a]
Na, %	0.55 ± 0.03	5.4	–	(0.5)
Sr, mg kg^{-1}	276.1 ± 24.8	9.0	4.6	264 ± 7
Zn, mg kg^{-1}	138.5 ± 7.9	5.7	5.8	147 ± 16

Mean ± *SD* Arithmetic mean and standard deviation for at least 4 determinations, *RSD* relative standard deviation, *Er* relative error

[a] Numbers in parentheses are informative values

Table 2 Concentrations of elements in sound and carious dentin tissues

Element	Sound dentin tissue		Carious dentin tissue	
	Mean ± SD	Range	Mean ± SD	Range
Ca (%)	26.1 ± 2.4	21.4–29.2	22.7 ± 4.4	10.6–30.8
Cl (%)	0.108 ± 0.006	1.00–0.11	1.28 ± 0.46	0.72–1.97
Mg (%)	0.549 ± 0.049	0.141–0.584	0.315 ± 0.078	0.242–0.438
Mn (mg kg^{-1})	0.52 ± 0.25	0.29–0.87	25.0 ± 7.8	16.5–37.6
Na (%)	0.66 ± 0.08	0.576–0.850	1.18 ± 0.22	0.814–1.53
Sr (mg kg^{-1})	174 ± 48	106–234	223 ± 36	172–255
Zn (mg kg^{-1})	119 ± 28	75–159	975 ± 704	456–2569

Mean ± SD Arithmetic mean and standard deviation of at least three determinations

Table 3 Concentrations of elements in sound and carious enamel tissues

Element	Sound enamel tissue		Carious enamel tissue	
	Mean ± SD	Range	Mean ± SD	Range
Ca (%)	32.2 ± 4.2	30.9–34.3	35.8 ± 3.3	32.7–40.7
Cl (%)	0.33 ± 0.53	0.247–0.392	0.90 ± 0.14	0.786–1.136
Mg (%)	0.182 ± 0.009	0.167–0.190	0.196 ± 0.008	0.184–0.208
Mn (mg kg^{-1})	1.00 ± 0.11	0.85–1.15	13.7 ± 8.4	6.9–26.8
Na (%)	0.68 ± 0.04	0.586–0.734	0.76 ± 0.14	0.59–0.88
Sr (mg kg^{-1})	187 ± 52	60.2–280.3	338 ± 64	286–444
Zn (mg kg^{-1})	186 ± 15	91–355	676 ± 257	347–1034

Mean ± SD Arithmetic mean and standard deviation of at least three determinations

for the elements Ca and Sr (for $p > 0.05$). In the carious dentin tissues Cl, Mn, Na and Zn concentrations were higher but Mg concentrations were lower than those found for sound dentins.

Results for enamel dental tissues showed no significant differences between sound and carious teeth for the elements Ca, Mg and Na ($p > 0.05$). For enamel tissues the concentrations of Cl, Mn, Sr and Zn were higher in carious teeth than those obtained for sound ones.

The concentrations of Ca obtained in enamel and dentin tissues indicated that this element is not related to incidence of caries. However, low concentrations of Mg in dentins could be related to prevalence of caries this tissue. Besides, higher concentrations of Zn found in teeth could be also related to incidence of caries. Harris et al. [4] also found increased concentrations of Zn in carious regions of dentine when compared with unaffected portions of the tooth by quantitative X-ray fluorescence imaging of sections of human teeth. On the other hand, our results obtained for Sr in enamel tissues did not agree with the ones obtained by Shashikiran et al. [1] that found higher concentration of this element in sound enamel than in carious enamel.

Comparisons made between the results obtained in dentin and enamel tissues of sound permanent teeth showed statistically significant differences for Ca, Cl, Mg, Mn and Zn ($p > 0.05$). Concentrations of Ca, Cl, Mn and Zn were higher in enamel tissues than those obtained in dentin ones, whereas these presented higher concentration of Mg.

Conclusions

The number of teeth analyzed in the present study was small. Within this small study we observed significant differences in several elements in dentin and enamel of carious and sound teeth. This fact indicates that trace elements in samples of these tissues have to be studied separately. These findings of the present study suggest more investigations on the correlation of trace elements with the prevalence of dental caries.

Acknowledgements The authors wish to thank FAPESP and CNPq from Brazil for financial support.

References

1. Shashikiran ND, Subba-Reddy VV, Hiremath MC (2007) Estimation of trace elements in sound and carious enamel of primary and permanent teeth by atomic absorption spectrophotometry: an in vitro study. Indian J Dent Res 18:157–162
2. Jalili VP, Tiwari A (1986) Fluorides and dental caries, a compendium, 1st edn. A publication of IDA, New Delhi
3. Dental Caries (2009) http//en.wikipedia.org/wiki/Dental_caries. Accessed 16 June 2009
4. Harris HH, Vogt S, Eastgate H, Lay PA (2008) A link between copper and dental caries in human teeth identified by X-ray fluorescence elemental mapping. J Biol Inorg Chem 13:303–306
5. Riyat M, Sharma DC (2009) Analysis of 35 inorganic elements in teeth in relation to caries formation. Biol Trace Elem Res. doi 10.1007/s12011-008-8305-6

6. Niedzielska K, Struzak-Wysokinska M, Wujec Z (1990) Analysis of correlation between the content of various elements in hard tissues in milk teeth with and without caries. Czas Stomatol 46:316–322
7. Curzon MJ, Losee FL (1977) Dental caries and trace element composition of whole human enamel: Eastern United States. J Am Dent Assoc 94:1146–1150
8. Tvinnereim HM, Eide R, Riise T, Fosse G, Wesenberg GR (1999) Zinc in primary teeth from children in Norway. Sci Total Environ 226:201–212
9. Burguera E, Romero Z, Burguera M, Burguera JL, Arenas H, Rondon C, Bernardi ML (2002) Determination of some cationic species in temporary teeth. J. Trace Elem Med Biol 16:103–112
10. Chew LT, Bradley DA, Mohd AY, Jamil MM (2000) Zinc, lead and copper in human teeth measured by induced coupled argon plasma atomic emission spectroscopy. Appl Radiat Isot 53: 633–638
11. National Institute of Standards & Technology, Standard reference material. 1486 Bone meal, Certificate of analysis, 1992