

## ALPHA AND BETA CASEIN IN DENTIN PROVED BY A METHOD OF TWO-DIMENSIONAL GEL ELECTROPHORESIS

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**Summary.** Being familiar with the organic structure of dentin tissue is of great importance for understanding the process of mineralization of extra-cellular dentin matrix. Using new technologies, of which two-dimensional gel electrophoresis stands out, a precise qualitative and quantitative analysis of the dentin structure is possible.

By applying this method on the dentin samples, the presence of alpha and beta casein has been proved. Caseins are phosphor proteins of remarkably sour character with strong affinity for binding calcium ions.

The discovery of alpha and beta casein in organic matrix represents an original and authentic contribution of this paper in determining the molecular structure of dentin. The role of casein in dentin is yet unknown and will be the subject of future researches. The tendency of positively electrified ions to bind points out the role of these complex proteins during dentin genesis and calcium ion transportation to mineralization front.

**Key words:** Dentin mineralization, alpha casein, beta casein, two dimensional gel electrophoresis

### Introduction

Being a specific hard dental tissue, dentin is rich in organic matter which comprises about 20% of its weight and 33% of its volume. Basic organic component of dentin is collagen. Nevertheless, non-collagen organic structure of dentin has not been sufficiently studied. Dentin and its compound ultra-structure, molecular structure and biochemical processes within this tissue have not been sufficiently cleared out and as such they represent a challenge for science. The role of non-organic dentin structure should be stressed in the process of the tissue mineralization. Revealing all the organic dentin constituents would help us understand dentin genesis. The essence of the dentin genesis process is the crystal hydroxyapatite mineralization of extra cellular dentin matrix. It is obvious that the biological classification takes place in a strictly defined environment in the presence of numerous organic macromolecules.

Introducing completely new technologies in the research of molecular structure of dentin tissue, such as two-dimensional electrophoresis gel, makes it possible to reveal the presence of the so far unknown proteins as parts of the dentin structure.

### Objective

The objective of this paper was to examine the qualitative and quantitative molecular dentin structure by using two-dimensional electrophoresis as well as to bring the achieved research results into correlation with the known literary data.

### The methodology of research

#### Sample preparation

Teeth of 100 grams in volume are frozen in the liquid azoth and smashed into very small pieces with hydraulic press into consistent powder. Frozen powdered teeth mass is treated with guanidine hydrochloride in 50 mM natrium acetate, with the pH value of 5.8. The objective of this procedure is to remove the cell remains and macromolecules which do not originate from dentin.

#### 2 D electrophoresis

Two-dimensional electrophoresis gel (2D electrophoresis) was first presented in 1975. It found its use in laboratory research of 2D electrophoresis by the end of the nineties. It was used within the research of the dentin structure for less than 2 years.

This procedure involves simultaneous systematic separation, identification and quantification of a large number of proteins from one sample of the examined substance. The name of the method itself points to a two-degree sample analysis (1).

The first dimension represents the separation of the protein within the sample by isoelectric focusing (IEF), while the second dimension represents standard SDS poly acryl amide gel electrophoresis. IEF is an electrophoresis method which separates proteins according to their isoelectric points (pI). It is known that the proteins are amphoteric molecules. They have positive, negative or zero electricity depending on the pH value of their environment.

The procedure is based on electrophoresis of the samples in the ready-made, already prepared gels with a pH gradient. The presence of the pH gradient is of great importance for IEF technique. Within the pH gradient, under the influence of the electric field, proteins are positioned on a place where their electricity is equal to zero. The proteins with positive electricity will migrate towards the cathode, becoming progressively less positively electrified while moving through the pH gradient until they reach their pI. On the other hand, the proteins with negative electricity will migrate towards the anode, becoming less negatively electrified until they reach zero electricity (2).

As soon as the process of isoelectric focusing has been finished (the first dimensions of 2D electrophoresis), the gel with "trapped" proteins is positioned on the top of the polyacrilamid gel (SDS PAGE) in a special instrument of Amersham company (Hoefer SE 600 – standard vertical). Electrical circuit is established and protein separation procedure is initiated on the basis of their molecular masses.

### Sample visualisation

Colouring protocol (Coomassie Blue Staining Protocol) during protein visualisation in SDS PGE entails plunging the gel into a solution of 50% ethanol and 10% octane acid within the time framework of at least one hour (3).

After that, the gel is plunged into water and then developed on a 0,04% formalin (35% formaldehyde in water) and 2% natrium carbonate during intensive gel mixing. Treated gels, coloured by Silver Staining technique, are being preserved on 4°C temperature in a 1% solution of octane acid, up to the moment when results analysis is being done (4,5).

As soon as the visualisation of the achieved electrophoresis results is finished, the points on the gel are chosen (after colouring, proteins can be seen as dark marks) which are then cut from the gel and sent to masspectrometric analysis.

Achieved masspectrometric analysis results in view of the determined sequences of amino-acids are then compared to the available data base in which the sequences of the discovered and described proteins are found. The analysis of the amino acid similarities is enabled by the use of special data base within which there are all the discovered amino acid chains as part of the polypeptides.

### The research results

The results of the two protein samples analysis achieved by the method of two-dimensional gel electrophoresis dentin fraction 23 show the presence of alpha and beta casein in dentin. The gel sections with proteins are masspectrometrically analyzed (Figure 1).

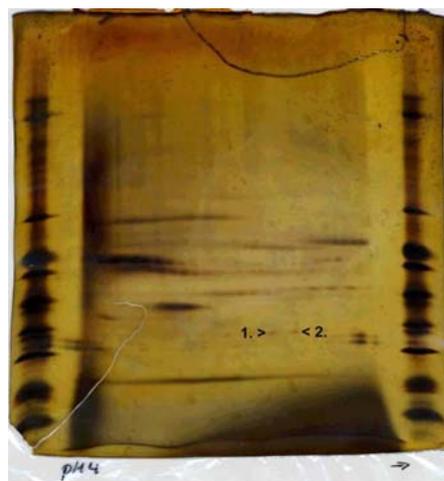
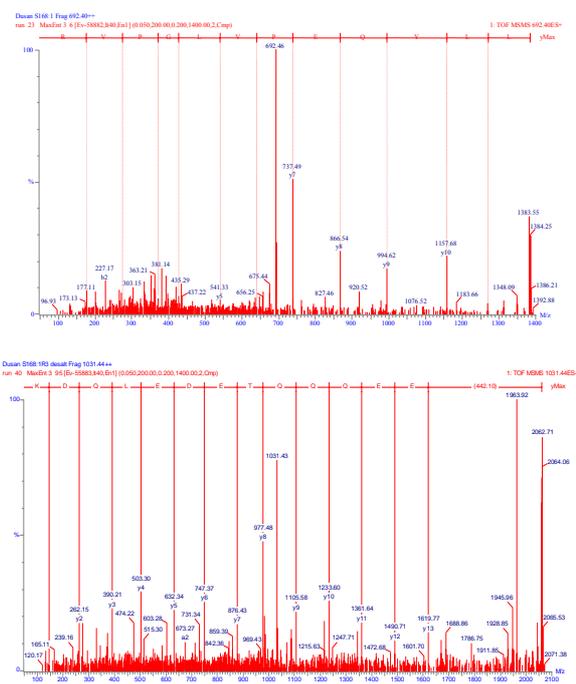


Fig. 1. Two-dimensional gel electrophoresis of dentin fraction 23. Pay attention on specimens in gel 1. and 2.

The results of masspectrometric analysis of the first sample are presented as a sequence of amino acids of two polypeptide chains which comprise macromolecule (Graphics 1 and 2).



Graph. 1 and 2. Mass spectrometry analyzing results of specimen 1. Aminoacid sequence viewed like two polypeptide chains

The achieved sequence of amino acids was compared to the data of the known proteins within the program BLAST NCBI data base. The tested protein sequence in the first sample presents beta casein (Figure 2 and 3).

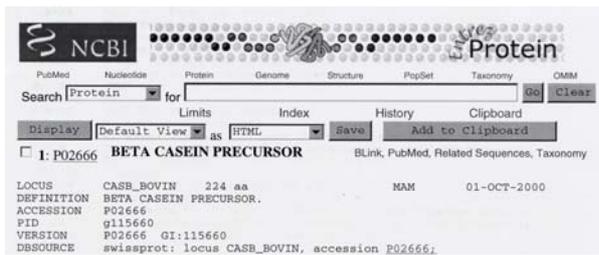


Fig. 2. Mass spectrometry results on specimen 1 of dentin gel fraction 23 viewed in BLAST program for protein amino acids sequences identification (NCBI). Specimen was recognized as BETA CASEIN

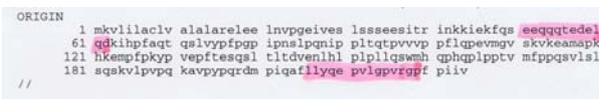
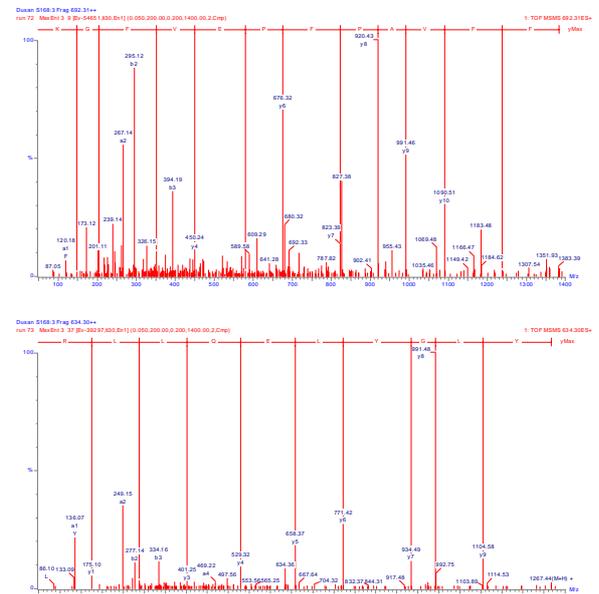


Fig. 3. Mass spectrometry results on specimen 2 of dentin fraction 23 viewed as sequence of amino acids (BETA CASEIN)

The mentioned procedure was used also for the analysis of the second sample achieved by two-dimensional gel electrophoresis dentin fraction 23. Massspectrometric analysis produced the following data about the sequence of amino acids which comprise the examined protein (Graphics 3 and 4).



Graph. 3 and 4. Mass spectrometry analysis of specimen 2. Sequences of amino acids were presented with two polypeptide chains

By comparing the results of massspectrometric analysis with the NCBI data base, the presence of alpha casein in organic dentin matrix has been proved (Figure 4 and 5).

**ALPHA CASEIN**

LOCUS 225632 214 aa MAM 21-OCT-1996 DEFINITION casein alphaS1. ACCESSION 225632 PID g225632 VERSION GI:225632 DBSOURCE prf: locus 1308122A; state: Alzheimer's disease; taxonomy: Mammalia. KEYWORDS alpha S1 Casein; Bovine; cDNA Cloning; Seq Determination; 1133bp/214AAs; Signal Seq of 15AAs; Codon Usage; Seq Homol with Guinea Pig's; Conserved in Noncoding Region.

Fig. 4. Mass spectrometry results on specimen 2 of dentin gel fraction 23 viewed in BLAST program of proteins amino acid sequences identification (NCBI). Specimen was recognized as ALPHA CASEIN

**ORIGIN**

1 mklliltclv avalarpkhp ikhggllpgev lnenllrfv apfpevfkgk  
kvnelksdig  
61 sesteqame dikemeaeasi sssgeivpns veqkhiqked  
vpserylgy l eqllrlkkyk  
121 vpqleivpns aeerlshsmke gidaqqkpep igvnqelayf  
ypelfrfqyq ldaypsgaw  
181 vylgtqytd apfsdipnp igsensekt mply

Fig. 5. Mass spectrometry results on specimen 2 of dentin gel fraction 23 viewed like amino acid sequences (ALPHA CASEIN)

**Discussion**

Casein represents a complex protein with a prosthetic group which contains phosphoric acid tied to some oxamic acid, mostly serine (6). Consequently, casein is a phosphor protein which is mostly found in the shape of soluble calcium casein salt. That is a protein of the acid character with isoelectric point between pH 4.5-4.7 (7). Prosthetic group of casein contains phosphoric acid tied to an OH group of oxamic acid, mostly serine and threonine in the shape of serine phosphate and threonin phosphate. Casein is a heterogeneous protein and can be found in many fractions – alpha, beta, gamma and cap. Fractions are mutually differentiated by the percentage of phosphor, isoelectric point, electrophoresis mobility and molecular weight (8)

Alpha casein is quantitatively the most important protein of casein complexity. It contains about 1% phosphor, whereas it doesn't contain cysteine and carbon hydrate (9). It is insoluble when the concentration is 0.03 M of calcium ions not only at 4 °C, but also at 25 °C, whereas when close to isoelectric point it is easily sedimented by calcium chloride. Molecular mass of alpha casein monomer is 23 kDa (6), which is in correlation with the results of our research.

Beta casein is different from the above mentioned casein by a slightly higher isoelectric point (pH 4.5). Its molecular mass is about 24.1 kDa (6), so that in this case a correlation between literary data and original research results is also present. Beta casein with calcium gives sediment at temperatures above 18°C (8).

Casein molecules could be tied into complex compounds of various connections among which we should point out not only hydrogenised and hydrophobic links but also those links created by the means of calcium and magnesium bridges (10).

The clear casein role in dentin genesis could only be anticipated. Taking into consideration the fact that casein is an acid phosphor protein, its explicit tendency to tie itself to calcium ions is evident (6). It is known that the calcium ion transport to the region where dentin genesis is being carried out is done by the acid phosphor proteins such as dentin phosphor protein, dentin sialoprotein and acid glycoprotein. Acid proteins which are a part of dentin have high affinity towards calcium ions (11,12).

In this way, it is possible to bring casein into connection with the process of dentin mineralization in the

sense of its importance for the transport and distribution of calcium ions in the mineralized front (12).

## Conclusion

The discovery of alpha and beta casein in organic matrix represents original and authentic contribution of this paper to the determination of molecular dentin structure. The role of casein in dentin is unknown and will be the subject of future researches.

For the time being we can only anticipate the importance of these complex proteins in the light of the fact that it is about acid phosphor proteins with explicit tendency to bind calcium ions.

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## ALFA I BETA KAZEIN U DENTINU DOKAZANI METODOM DVODIMENZIONALNE GEL ELEKTROFOREZE

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*Kratak sadržaj: Poznavanje organske strukture dentinskog tkiva je od velikog značaja za razumevanje procesa mineralizacije ekstracelularnog matriksa dentina. Korišćenjem novih tehnologija, od kojih se ističe dvodimenzionalna gel elektroforeza, moguća je precizna kvalitativna i kvantitativna analiza sastava dentina.*

*Primenom ove metode na uzorcima dentina dokazano je prisustvo alfa i beta kazeina u ovom tkivu. Kazeini su fosfoproteidi izuzetno kiselog karaktera, sa jakim afinitetom za vezivanje jona kalcijuma.*

*Otkriće alfa i beta kazeina u organskom matriksu predstavlja originalni i autentični doprinos ovog rada u determinaciji molekularne strukture dentina. Uloga kazeina u dentinu je nepoznata i biće predmet budućih istraživanja. Sklonost ka vezivanju jona sa pozitivnim naelektrisanjem ukazuje na izvesnu ulogu ovih složenih proteina tokom dentinogeneze i transporta jona kalcijuma do mineralizacionog fronta.*

*Ključne reči: Mineralizacija dentina, alfa kazein, beta kazein, dvodimenzionalna gel elektroforeza*