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Note

Gel chromatography of proteins from human teeth

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In recent years, much attention has been devoted to the study of various protein components of human teeth. The main protein constituent, collagen, which represents about 90% of the organic matrix, has been extensively described by Höhling[1]. Nevertheless, there are several other proteins that might have significant biological roles, including, as pointed out by Veis and Spector[2], a phosphoprotein fraction, found in bovine dentine. The relationship of the physical and chemical properties of the hard tooth tissues, enamel and dentine, to those of their protein constituents has been studied by a number of workers, using various techniques including gel and ion-exchange chromatography[3–6].

This paper describes the separation of the protein components of the organic matrix of human teeth by chromatography on different Sephadex gels. The fractions obtained may serve as useful starting materials for further isolation and characterization of these proteins.

MATERIALS AND METHODS

Human teeth were demineralized for about 10 weeks in 10% nitric acid. The rubbery remains of the teeth were homogenized in the same solution in an ordinary glass homogenizer with a PTFE piston. The resulting homogenate was then filtered through a coarse sintered-glass filter and brought to pH 11 by adding 0.2 *M* sodium hydroxide solution. The resulting yellowish solution was subsequently neutralized to pH 7 with 1 *M* hydrochloric acid. The heavy white precipitate that formed during neutralization was separated by filtration through a sintered-glass filter.

The white precipitate was washed on the filter with water, then dissolved in 5% acetic acid and freeze-dried. The solution of the resulting white powder showed no biuret reaction and its ultraviolet absorption spectrum was not characteristic of proteins. It was therefore concluded that the precipitate contained no substantial amount of proteins.

The yellow filtrate, however showed a pronounced biuret reaction, indicating the presence of proteins, and its absorption spectrum in the ultraviolet region closely

resembled those of protein solutions in exhibiting an absorption band with a maximum at 280 nm and a steep rise in absorbance below 250 nm. It was therefore considered likely that this neutral solution of the yellow filtrate contained a major part of the collagen and other proteins from the organic matrix of human teeth.

The low-molecular-weight compounds and the remains of inorganic material were removed from the concentrated yellow solutions of proteins by gel filtration on a 19 × 2 cm Sephadex G-25 column. Chromatography was carried out with 0.01 *M* Tris–hydrochloric acid buffer (pH 7.4) using a fraction collector. The absorbance of the eluate was measured at 280 nm by means of a spectrophotometer (Carl Zeiss Jena, Jena, G.D.R.). Two pronounced and well separated peaks were obtained. The first, high-molecular-weight, peak showing a strong biuret reaction and an absorption spectrum characteristic of proteins. However, the absorption spectrum of the second, low-molecular-weight, peak differed from the usual protein spectrum in showing a strong absorption band with a maximum at 300 nm and a shallow minimum at 260 nm. Subsequent re-chromatography of both compounds on the same column under the conditions described above showed that both compounds were reasonably pure and devoid of compounds contained in the other peak. The material from the first peak, designated "tooth proteins", was freeze-dried and used for further experiments. It formed a light, slightly yellowish white powder.

The tooth proteins obtained in the procedure described above were fractionated by gel chromatography on various types of Sephadex. The columns, of dimensions 100 × 1 cm, of Sephadex G-50, G-75, G-100, G-150 and G-200 gels were prepared according to the method of Flodin and Killander[7] in 0.01 *M* Tris–hydrochloric acid (pH 7.4). The same buffer was used for elution. Fractions of 1 ml of the eluent buffer were collected on a fraction collector (Fractiomat, Budapest, Hungary). The

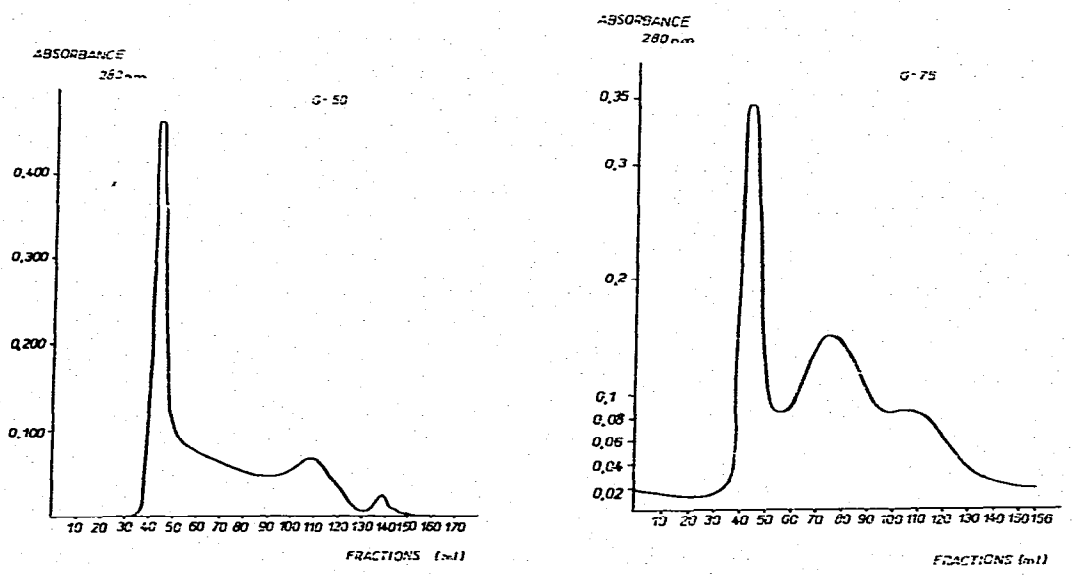
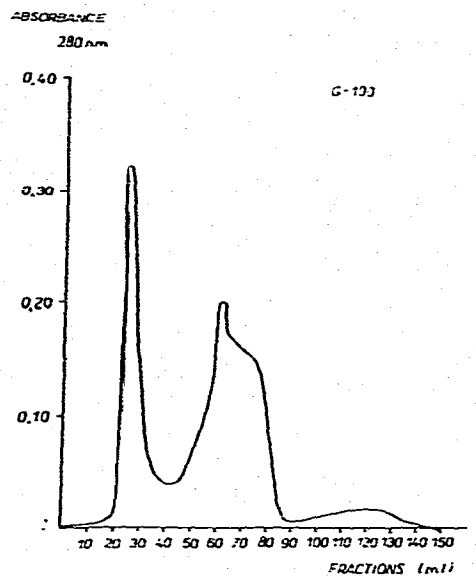


Fig. 1. Separation of human tooth proteins on a Sephadex G-50 column. Absorbance at 280 nm of fractions flowing out of the chromatographic column.

Fig. 2. Separation of human tooth proteins on a Sephadex G-75 column.



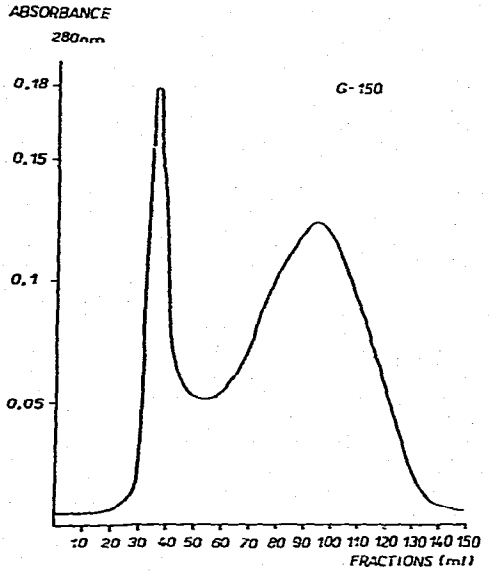


Fig. 3. Separation of human tooth proteins on a Sephadex G-100 column.

Fig. 4. Separation of human tooth proteins on a Sephadex G-150 column.

freeze-dried tooth proteins were dissolved in the above buffer to form a 20% (w/v) solution, and 0.5 ml of this solution was applied to the Sephadex column and subjected to gel chromatography. The absorbance at 280 nm of each 1-ml fraction was measured against the buffer in a Zeiss spectrophotometer.

RESULTS AND DISCUSSION

The results are summarized in Figs. 1–5. The pattern of the separation of tooth proteins on Sephadex G-50, shown in Fig. 1, suggests that, in addition to the real

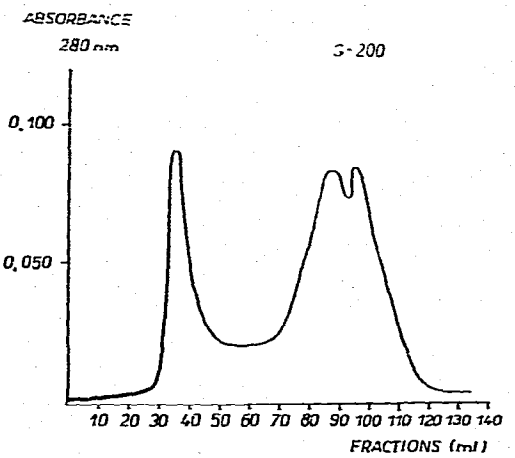


Fig. 5. Separation of human tooth proteins on a Sephadex G-200 column.

protein fraction in the initial sharp peak, various peptides and other compounds of low molecular weight are present in the long, undifferentiated slope that follows the protein peak. The same pattern occurs in the separation on Sephadex G-75 (Fig. 2).

The presence of these peptides can probably be ascribed to the hydrolytic action of the strong acid used in the demineralization of the teeth. The slightly yellowish colour of the tooth proteins is probably due to the action of nitric acid during the demineralization process (xanthoproteic reaction).

Chromatography on Sephadex G-100 (Fig. 3) and also on Sephadex G-150 and G-200 (Figs. 4 and 5) showed a clear separation of tooth proteins into two peaks. The comparatively large amount of proteins in the first peak (Fig. 5, left) in chromatography on Sephadex G-200 shows that approximately half of the proteins contained in teeth are of rather high molecular weight (> 200,000). The other half of the proteins contains a number of different protein fractions of varying molecular weight, as shown in chromatography on Sephadex G-100 and G-150 (Figs. 3 and 4). The proteins from these peaks, after collection and concentration, would be suitable for study, *e.g.*, by polyacrylamide gel electrophoresis, and as a suitable starting material for further purification.

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