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PROTEOLYTIC ACTIVITY OF DENTAL PLAQUE MATERIAL

VII. SEPARATION OF PROTEINASES FROM DENTAL PLAQUE MATERIAL WITH THE AID OF GEL FILTRATION THROUGH SEPHADEX AND AGAROSE COLUMNS

by

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INTRODUCTION

The presence of the dental plaque has been shown to constitute a primary etiologic factor in caries (*Stephan,* 1938, 1940; *Strålfors,* 1948, 1950) as well as in periodontal disease (*Bibby,* 1953; *Schei et al.,* 1959; *Björn,* 1960; *Ramfjord,* 1961; *Greene,* 1963; *Brandtzaeg,* 1964; *Koch & Lindhe,* 1965).

Many different properties of the dental plaque has been studied in the past such as acid production (*Stephan,* 1944), urease activity (*Frostell,* 1960), toxic products (*Rosebury,* 1952), allergic reactions (*Mergenhagen,* 1960), and enzymatic reactions (*Schultz-Haudt et al.,* 1954, 1955).

The proteolytic activity in the dental plaque material may have some relationship to the pathogenesis of periodontal disease which is a morbid condition including inflammatory pro-

cesses in the tooth supporting structures. Proteolysis also may play a role in dental caries especially in dentin caries.

In six previous articles (*Söder & Frostell,* 1966; *Söder,* in press; *Söder, Lundblad & Lindqvist,* 1966) the proteolytic activity of dental plaque material after high and low speed centrifugation was studied and the relative activity in the pellet and supernatant was compared to the total proteolytic activity. The effect of pH, inhibitors and activators was studied (*Söder, Lundblad & Lindqvist,* 1966; *Söder,* in press) on proteinases purified through Sephadex G-100, G-200 columns and zone-electrophoresis in Sephadex G-25, according to methods given by *Porath & Flodin* (1959), *Flodin* (1962), *Gelotte* (1964).

One of the enzyme fractions obtained was suspected of containing more than one enzyme and the first proteinase was obviously not pure. The present communication deals with the further separation of dental plaque proteins and proteinases with the aid of more advanced methods employing Sephadex and the material agarose which separates (*Lundblad & Schilling,* 1966) proteins by different properties than Sephadex. Some of the properties of the enzymes were studied.

MATERIAL AND METHODS

Dental plaque material was collected from persons with clinically normal or diseased gingiva and was treated as described in earlier articles (*Söder & Frostell,* 1966; *Söder,* in press). The dry weight of the material was determined, as earlier described. To the plaque material was added 3.0 ml 0.1 M Tris(hydroxymethyl) aminomethane (TRIS)-HCl buffer pH 8.1 or 9.1. The crude plaque suspension was ground in a Virchow glass mortar in order to break up the bacterial aggregations without destroying the bacterial cells. The suspensions were centrifuged at 2500 × g for 15 minutes (Original Wifug, Type XI, 4 × 100) and the supernatant was divided in two parts. One part was put in a Visking tube and concentrated against dextrose. This concentrate was used for gel filtration. The other part of the supernatant was used to determine the initial activity. All the experiments were carried out at room temperature.

Some of the experiments were performed with pooled plaque material from several persons and other experiments with material from only one person.

All the experiments were performed as soon as possible after the dental plaque material was collected from the patient.

Gel filtration

Sephadex G-100, G-200 and G-200 superfine (Pharmacia, Uppsala, Sweden) and agarose 2 and 4 per cent* were used to fractionate the dental plaque material. The Sephadex gels were suspended in 0.2 per cent NaCl and 2 per cent butanol and were allowed to swell 4 or 5 days at room temperature. Incomplete swelling before application in the columns gave a very slow flow rate. Agarose 2 and 4 per cent were received in suspensions and since further swelling was unnecessary it was used directly.

The gels were carefully added to the columns and the medium was continuously flushed with 0.1 M TRIS-HCl buffer, pH 8.1 or 9.1, in 0.5 M NaCl containing 1.0 per cent butanol as bactericidal agent. This procedure was continued for 2 or 3 days in order to wash the gels.

A thin layer of Sephadex G-25 was added on top of the gel to prevent any disturbance when the samples were added. The column was completely equilibrated as judged by the elution volume over a certain time interval.

The dental plaque material was adjusted to a volume of 2.0 ml with the corresponding buffer and was applied with a special syringe to the top of the gel. The elution was performed at room temperature in the buffer mentioned above and fractions of a desired volume were collected in a fraction collector equiped with a device for maintaining constant volume.** The elution of the proteins was followed continuously by measuring the UV absorption at 254 mμ with a LKB Uvicord Monitor and a recording meter. The protein contents of the fractions were estimated also

* Agarose 2 and 4 per cent were obtained from Docent B. Gelotte and Dr. M Joustra, AB Pharmacia, Uppsala, Sweden, to whom the authors wish to express their gratitude.

** Constructed by Eng. L.-G. Falksveden, Chemistry department, Natl. Bact. Lab., Stockholm, Sweden.

from absorption measurements at 220, 260 and 280 mμ in a Beckman DU spectrophotometer with 1 cm quartz cuvettes. Effluent volumes in the figures are given in ml.

The protein and nucleic acid content in the fractions were determined using nomograms as discribed by *Warburg & Christian* (1942).

The sizes and other data of the Sephadex and agarose columns are given in the figure legends.

Assay of the proteolytic activity

The proteolytic activity of the various fractions were tested on two natural substrates, gelatin (U.S.P. gran., Fischer, Sc. Co., N.J., U.S.A.) and hemoglobin (Difco lab., Michigan, U.S.A.) and one synthetic substrate, α-N-benzoyl-L-arginine ethyl ester-HCl (BAEE) (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) (*Brown,* 1960; *Forde et al.,* 1962).

Gelatin splitting activity was measured by changes in the viscosity of gelatin according to the method of *Hultin* (1946, 1948), *Lundblad* (1962) and *Lundblad & Hultin* (1966) employed in a previous paper (*Söder,* in press).

One ml of the dental plaque extract was mixed with 3.0 ml of a 4 per cent gelatin solution in 0.1 M TRIS-HCl buffer, pH 7.5 or 8.5, containing merthiolate as bactericidal agent. The outflow times of the mixtures in Ostwald viscosimeters were measured and the proteolytic activity was calculated according to *Hultin*'s formula. The value so obtained was multiplied by 10^9 and called *Hultin* units (H.U.) (*Lundblad,* 1962).

Hemoglobin splitting activity was measured by the method of *Anson* (1938), using urea denatured bovine hemoglobin. The reaction mixture consisted of 1.0 ml of the dental plaque extract, 2.0 ml 0.2 M TRIS-HCl buffer, pH 7.5 or 8.5, and 1.0 ml of a 4 per cent hemoglobin solution. The mixture was incubated at 37° C for various periods of time.

The enzymatic reaction was stopped with 5.0 ml 0.3 M trichloroacetic acid (TCA) and the mixture filtered. The amount of amino acid residues released was determined spectrophotometrically at 280 mμ and expressed as equivalent amounts of tyrosine.

Experiments were run also with BAEE as substrate and its hy-

drolysis was measured by titration of the liberated hydrogen ions with NaOH with the aid of an automatic titrator Radiometer TTT (Copenhagen, Denmark) which maintained the pH constant (*Smith & Parker,* 1958).

The reaction mixture consisted of 1.0 ml 0.02 M BAEE and 5.0 ml $3 \cdot 10^{-3}$ M boric acid—borax buffer and 4.0 ml 0.01 M $CaCl_2$ (pH 8.0) and 1.0 ml of the dental plaque extract or 1.0 ml distilled water as control. The amount of BAEE remaining at the end of the experimental period was determined as described earlier (*Söder,* Part IV, in press) by addition of 0.5 ml 0.01 per cent trypsin (3 times cryst.) solution and titrated as described above. The titration value was compared to a standard titration of BAEE with 0.01 per cent trypsin under defined conditions. From the difference between the initial amount of BAEE and the amount remaining it was possible to calculate the enzymatic activity.

EXPERIMENTS AND RESULTS

When pooled samples were run on different gel columns containing either Sephadex G-100, G-100 superfine, G-200 or G-200 superfine (Fig. 1), comparable curves were obtained when the optical density was determined at 260 mμ and 280 mμ. The curves in figure 1 are representative of more than 100 separations of dental plaque material on different Sephadex gels. As can be seen there were always two main protein peaks. At 220 mμ another peak was observed between the two main ones (Fig. 1 b and c) which was not apparent in the other wavelengths used. Sephadex G-200 superfine (Fig. 1 c) gave the best separation of the three peaks.

When samples obtained from only one patient was analyzed with G-100 the same protein peaks were found (Fig. 2), but the middle peak visible at 220 mμ was close to the high molecular protein peak and could not be separated from it. The figure is representative of more than 20 experiments of this kind. From the figure it is apparent also that the protein concentration in the first peak was greater than in the second and in both a comparatively small amount of nucleic acids were present.

The distribution of proteolytic activity within the various fractions of pooled dental plaque extracts after separation on Sephadex G-100 or G-200 is shown in Fig. 3. Only two enzyme peaks

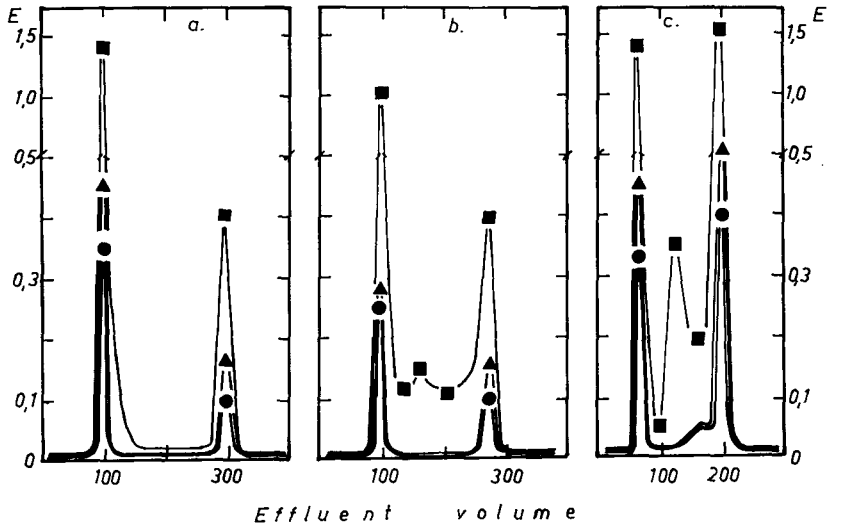

Fig. 1. Gel filtration chromatogram of pooled dental plaque supernatant after centrifugation at 2500 × g. The absorption curves represent extinction (E) readings at: ■——■ = 220 mμ, ▲——▲ = 260 mμ, ●——● = 280 mμ.
a) 3.0 ml dental plaque extract (4.2 mg protein) was applied onto a 1.5× 170 cm Sephadex G-100 column and eluted with 0.1 M TRIS-HCl buffer (pH 8.1) 0.5 M NaCl and 1 per cent butanol. Flow rate 7.0 ml/hour at room temperature.

b) 2.0 ml dental plaque extract (3.2 mg protein) was applied onto a 1.5× 165 cm Sephadex G-200 column and eluted with the same buffer as a) at pH 8.2. Flow rate 6.0 ml/hour at room temperature.

c) 2.0 ml dental plaque extract (3.4 mg protein) was applied onto a 1.5× 165 cm Sephadex G-200 superfine column and eluted with the same buffer as a) at pH 9.0. Flow rate 0.8 ml/hour at room temperature.

were found (called Protease I and II) when either gelatin or hemoglobin was used as substrate. Similar results were obtained when extracts from single persons were analyzed (Fig. 4).

Experiments with 4 per cent agarose

A series of experiments with 4 per cent agarose were carried out on pooled dental plaque material or material from one patient (Fig. 5 a, b, c, d). The two main protein peaks were still apparent. The gelatinolytic activity was determined immediately

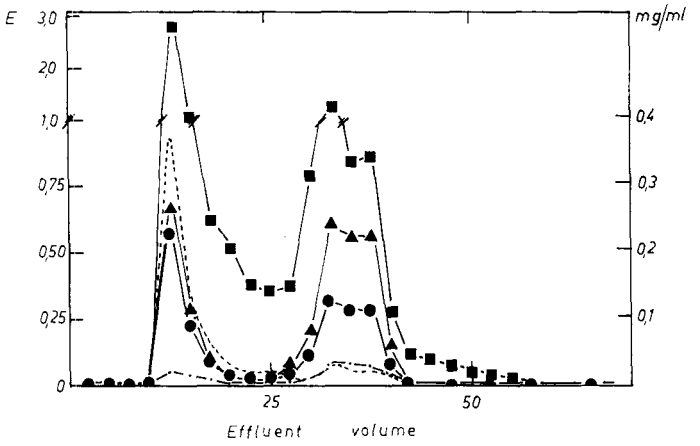

Fig. 2. Dental plaque extract concentrate obtained from one person was filtered through a 0.60×160 cm Sephadex G-100 column. 2.0 ml extract (5.4 mg protein) was applied and eluted with 0.1 M TRIS-HCl, pH 8.1. Flow rate 3.75 ml/hour at room temperature. Fractions collected in 2.5 ml volumes. Extinction coefficients read at: ■——■ = E_{220}, ▲ - -▲ = E_{260}, ●——● = E_{280}, - - - - - = mg protein/ml, —.—.—.— = mg nucleic acid/ml.

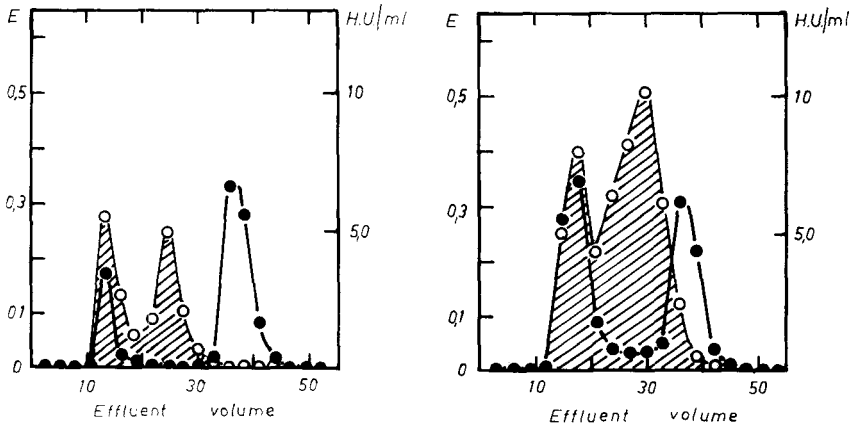

Fig. 3. The distribution of proteolytic activity in gel filtration fractions of concentrated pooled dental plaque extract.

a) 2.0 ml of the extract (3.1 mg protein) was applied to a 0.60×170 cm column of Sephadex G-100 and eluted with 0.1 M TRIS-HCl buffer (pH 8.1), 0.5 M NaCl and 1 per cent butanol. Flow rate 4.0 ml/hour at room temperature. ●——● = E_{280}, o///o = gelatinolytic activity expressed in H.U./ml.

b) 2.0 ml dental plaque extract (3.8 mg protein) applied to a 0.60×160 cm Sephadex G-200 column and eluted with the same buffer, pH 8.1. Flow rate 3.5 ml/hour. ●——● = E_{280}, o///o = gelatinolytic activity in H.U./ml.

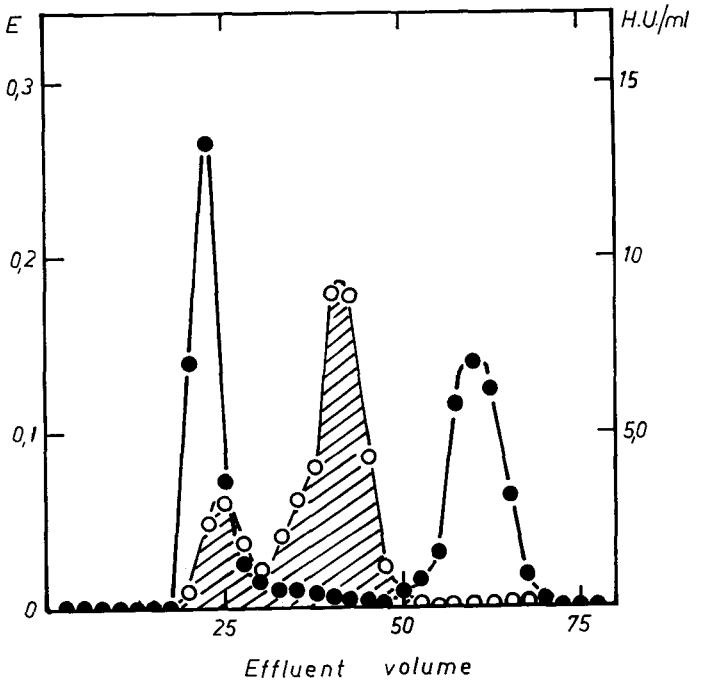

Fig. 4. Dental plaque extract obtained from one patient (4.1 mg protein) was filtered through a 0.64×160 cm Sephadex G-100 superfine column and eluted with 0.1 M TRIS-HCl buffer (pH 8.1), 0.5 M NaCl and 1 per cent butanol. Flow rate 3.5 ml/hour at room temperature.
●——● = E_{280}, o///o = H.U./ml.

after fractionation and after one day storage at 5° C; no significant loss of activity was observed (Fig. 5 a).

When hemoglobin was used as substrate (Fig. 5 b) a greater enzyme activity and sensitivity was obtained at pH 7.5 than at 8.5.

In some experiments an additional enzyme fraction (Protease III) was clearly visible following the second protein peak when either hemoglobin or gelatin was used as substrate (Fig. 5 c and d). This peak appeared when either material obtained from one or several patients were used.

Parallel experiments with 2 and 4 per cent agarose and Sephadex G-100 and G-200

Parallel separations through columns containing either 2 or 4 per cent agarose or Sephadex G-200 gave the same protein peaks

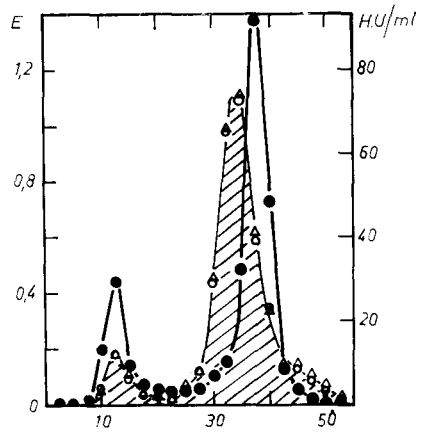

Fig. 5 a.

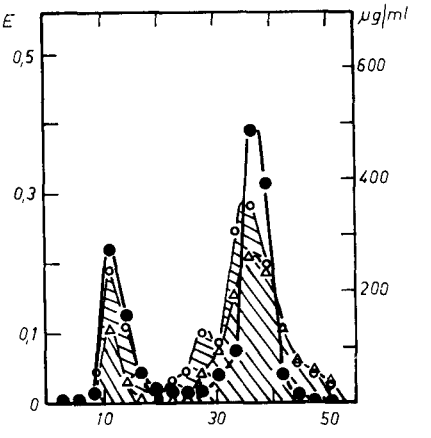

Fig. 5 b.

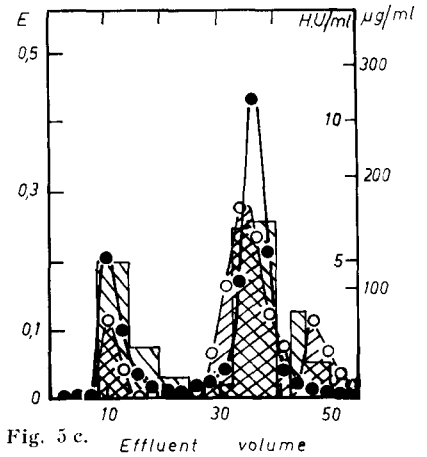

Fig. 5 c.

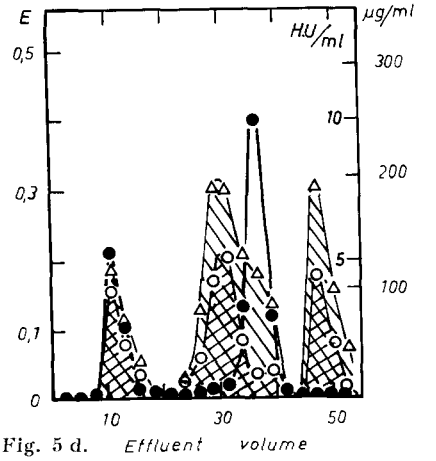

Fig. 5 d.

Fig. 5. Gel filtration chromatogram of dental plaque low speed centrifugation supernatant passed through a 0.60×160 cm 4 per cent agarose column and eluted with 0.1 M TRIS-HCl buffer pH 9.0, 0.5 M NaCl and 1 per cent butanol at room temperature.

a) 1.4 ml pooled plaque extract (10.5 mg protein) was fractionated at a flow rate of 1.0 ml/hour. The gelatinolytic activity was determined immediately and after storage at 5 °C for a one day interval and expressed in H.U./ml. △///△ = first day, o///o = second day, ●——● = E_{280}.

b) 2.0 ml pooled plaque extract (8.1 mg protein) was fractionated at a flow rate of 0.9 ml/hour. The hemoglobinolytic activity expressed as tyrosine equivalents was determined at pH 7.5. = o ▨ o and pH 8.5 = △ ▨ △ for 20 hours at 37°C. ●——● = E_{280}.

c) 1.25 ml pooled plaque extract (8.2 mg protein) fractionated at a flow rate of 0.8 ml/hour. The gelatinolytic and the hemoglobinolytic activity were determined and expressed as H.U./ml (o///o) and µg/ml tyrosine (▨), respectively, after 20 hours incubation at 37°C. ●——● = E_{280}.

d) 1.5 ml plaque extract from one person (7.6 mg protein) was fractionated at a flow rate of 0.9 ml/hour. The gelatinolytic activity and hemoglobinolytic activity were measured as H.U./ml (o///o) and µg/ml tyrosine (△ ▨ △), respectively. ●——● = E_{280}.

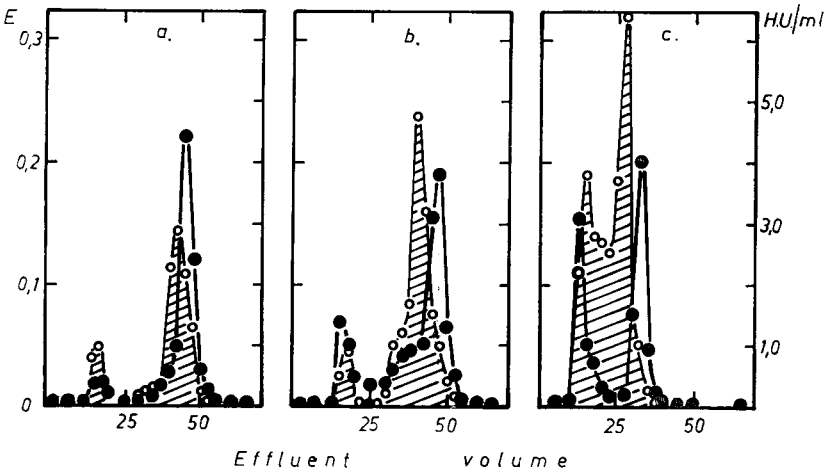

Fig. 6. Parallel gel filtration chromatograms of pooled dental plaque extracts passed through a 0.60×170 cm column of 2 or 4 per cent agarose or Sephadex G-200. ●——● = E_{280}, o///o = gelatinolytic activity expressed as H.U./ml. The eluent was 0.1 M TRIS-HCl buffer (pH 8.1), 0.5 M NaCl and 1 per cent butanol. Fractions were collected in 2.5 ml volumes. In contrast to previous experiments the gelatinolytic activity was determined by mixing 2.0 ml of each fraction with 2.0 ml of a 6 per cent gelatin stock solution in 0.5 M TRIS-HCl buffer (pH 8.1).

a) 1.5 ml plaque extract (2.7 mg protein) was fractionated on 2 per cent agarose. Flow rate 2.3 ml/hour.

b) 1.5 ml plaque extract was fractionated on 4 per cent agarose with a flow rate of 1.85 ml/hour.

c) 1.5 ml plaque extract was fractionated on Sephadex G-200 with a flow rate of 1.85 ml/hour.

as before (Fig. 6). The proteins in the first peak from 2 per cent agarose separation had a molecular weight over 10^6 (*Gelotte*, 1966) and the first enzyme activity observed still coincided with this peak. The total enzymatic activity (Protease I and II), however, was higher when the dental plaque extract was separated through Sephadex G-200 than through agarose (compare figure 6 a and c).

Aliquots from the same pooled plaque material were separated through 2 and 4 per cent agarose and Sephadex G-100. Here, the third enzyme peak (Protease III) was visible when 2 per cent agarose was used (fraction 55—60 ml in figure 7 a) and more

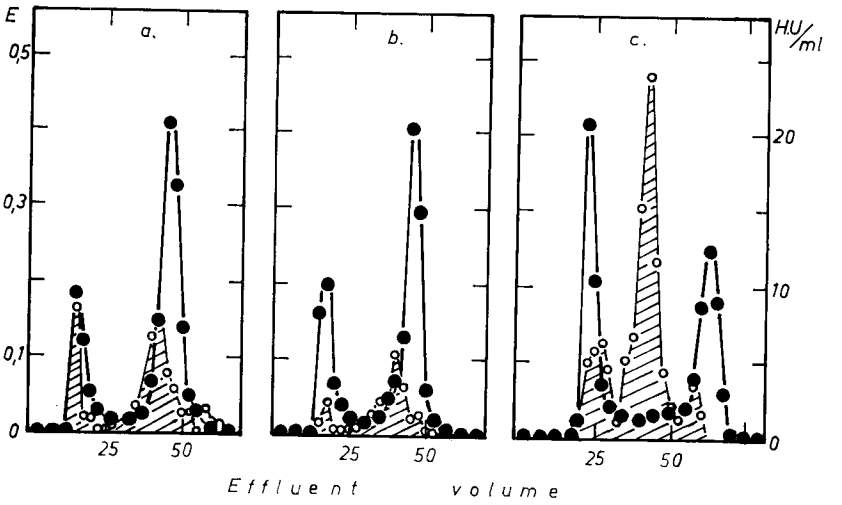

Fig. 7. Parallel separation of dental plaque extract on either 2 or 4 per cent agarose of Sephadex G-100. ●——● = E_{280}, o///o = H.U./ml. Fractions were collected in 2.5 ml volumes.

a) 2.0 ml extract (8.8 mg protein) was fractionated on a 2 per cent agarose 0.60×170 cm column at a flow rate of 2.0 ml/hour.

b) 2.0 ml extract (8.8 mg protein) was fractionated on a 4 per cent agarose 0.60×170 cm column at a flow rate of 1.85 ml/hour.

c) 2.0 ml extract (8.8 mg protein) was fractionated on a Sephadex G-100 superfine 0.64×170 cm column at a flow rate of 1.85 ml/hour.

clearly with G-100 (fraction 55—60 ml in figure 7 c). The first enzyme peak was partly separated from the first protein peak in fraction 25—32 ml figure 7 c.

When both gelatinolytic and hemoglobinolytic activity were tested in the separated fractions of pooled plaque material from 2 per cent agarose and Sephadex G-100 superfine, hemoglobinolytic activity was found in a broader second enzyme peak (fraction 35—50 ml in figure 8 a) than gelatinolytic (fraction 38—45 ml). The third enzyme peak (Protease III) was not evident after fractionation with 4 per cent agarose (Fig. 8 a) but appeared after Sephadex G-100 superfine fractionation and contained activity against both gelatin and hemoglobin (fraction 57—62 ml in figure 8 b). The third enzyme fraction was present in the second low molecular protein peak (fraction 50—70 ml in figure 8 b).

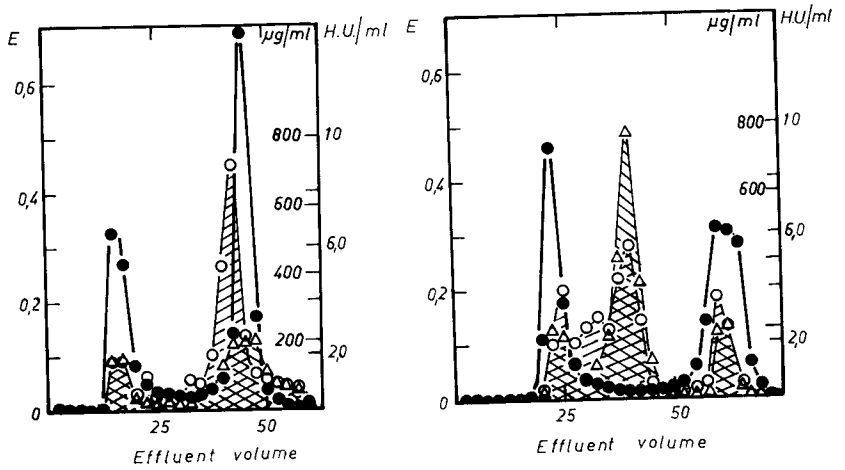

Fig. 8 a. Fig. 8 b.

Fig. 8. Parallel separation of pooled dental plaque extract on a 4 per cent agarose 0.60×170 cm column and Sephadex G-100 superfine in a 0.64×170 cm column. ●——● = E_{280}, o///o = H.U./ml, △\\\△ = µg tyrosine/ml. Fractions were collected in 2.5 ml volumes.

a) 2.0 ml extract (8.8 mg protein) was fractionated on 2 per cent agarose at a flow rate of 1.94 ml/hour.

b) 2.0 ml plaque extract was fractionated on Sephadex G-100 superfine at a flow rate of 2.29 ml/hour.

The absorption spectra of various fractions within the two protein peaks, from separation on G-100 superfine, are shown in figure 9. The curve for the first peak showed a broad plateau of absorbance probably dependent on the particulate nature of the fractions (Fig. 9 a). The second peak, however, showed the possible presence of nucleic acids and aromatic amino acids (Fig. 9 b and c) (*Chargaff & Davidsson*, 1955).

The proteolytic activity in fractions isolated from pooled dental plaque material filtered through Sephadex G-100 superfine was tested against hemoglobin and BAEE as substrates and the results are shown in figure 10. The measurement of hemoglobinolytic activity was very sensitive and the third enzyme was clearly visible here (fraction 55—68 ml in figure 10 a). In this experiment the hemoglobinolytic activity of Protease I was only slightly higher than Protease III. Protease II was twice as active against BAEE as Protease I.

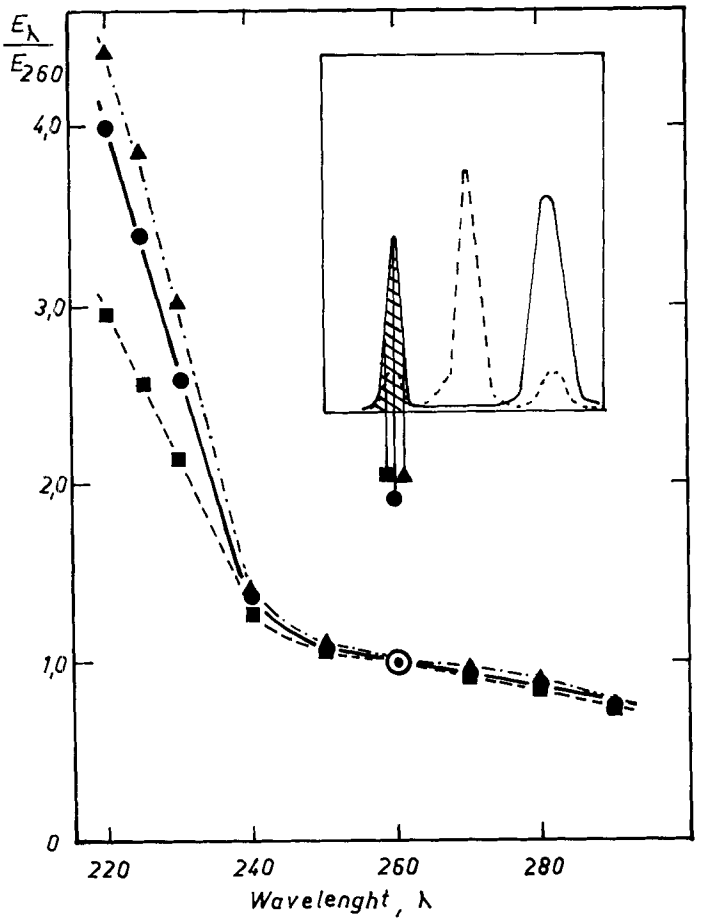

Fig. 9 a.

Fig. 9. Ultraviolet absorption spectra of the two protein peaks after separation of pooled dental plaque extract on Sephadex G-100 superfine. a) UV absorption spectra of fractions taken within the first high molecular protein peak. ●——● = the fraction taken at the maximum of the first protein peak. ■- -■ = a fraction taken at the beginning of the first protein peak. ▲– • – ▲ = a fraction taken at the end of the first protein peak. The absorption at 260 mμ = 1.

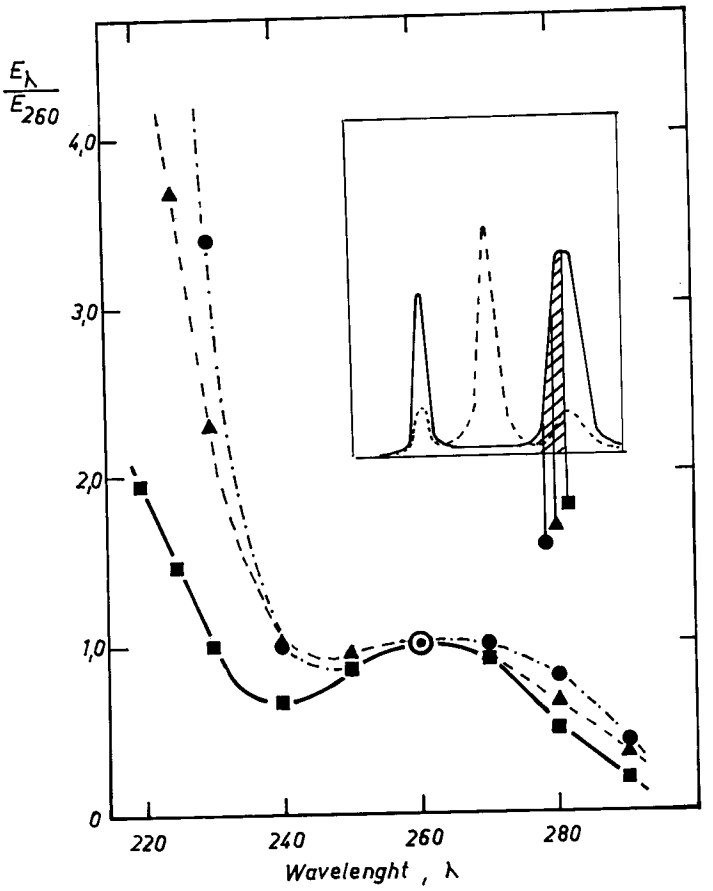

Fig. 9 b.

UV absorption spectra of various fractions within the last protein peak.

The relation between the amount of nucleic acids and dental plaque proteins in the experiments are shown in figure 10 b. Most of the proteins appeared in the first protein peak while both peaks contained about the same amount of nucleic acids.

The percentage of proteins recovered after separation on 2 and 4 per cent agarose and Sephadex G-100 from the total amount of the proteins in the original dental plaque sample are shown in figure 11. The figure shows that about half of the proteins were recovered after separation through the gels.

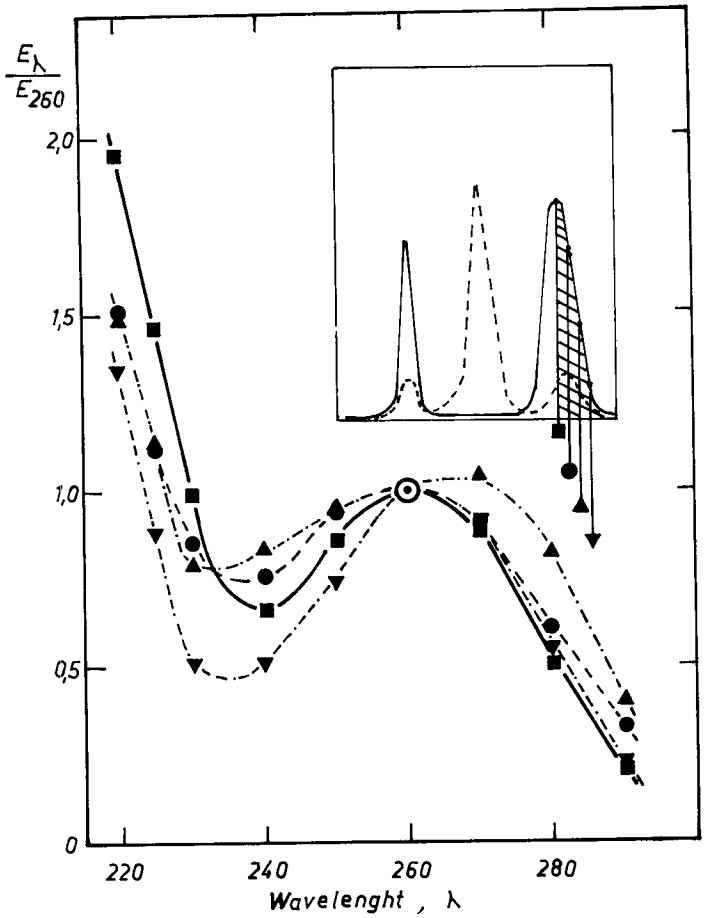

Fig. 9 c.

UV absorption spectra of various fractions within the last protein peak.

DISCUSSION

The fractionation of the low speed supernatant of dental plaque material through 2 or 4 per cent agarose or Sephadex G-100, G-100 superfine, G-200 and G-200 superfine revealed two main protein peaks in agreement with previous results (*Söder, Lundblad & Lindqvist,* 1966). The first peak contained high molecular proteins (Fig. 1, 6, 7). An additional peak between the two main ones was visible at 220 mμ, which could be partly separated from the first peak by use of Sephadex G-200 superfine (Fig. 1 c).

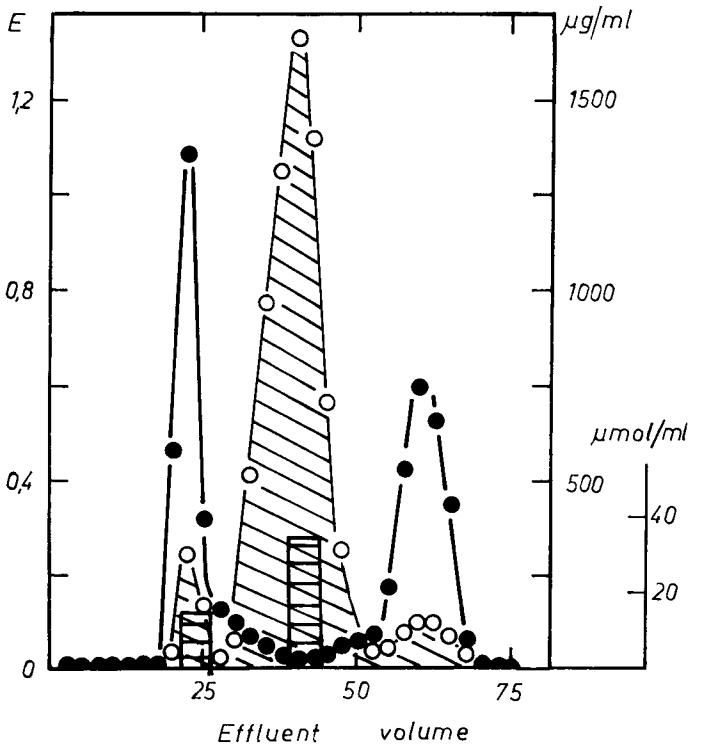

Fig. 10 a.

Fig. 10. Separation of pooled dental plaque extract on Sephadex G-100 superfine in a 0.64×170 cm column. The protein content was 10.2 mg.
a) ●——● = E_{280}, o ⋘ o = μg tyrosine/ml, ▤ = μmol. BAEE/ml. The hydrolysis of hemoglobin was measured at 37°C for 20 hours and BAEE hydrolysis at room temperature for 20 hours.

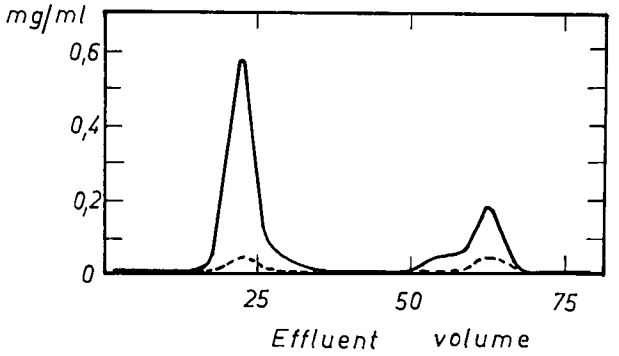

Fig. 10 b.

b) ——— = mg protein/ml and - - - = mg nucleic acid/ml in the fractions from experiments shown in figure 10 a.

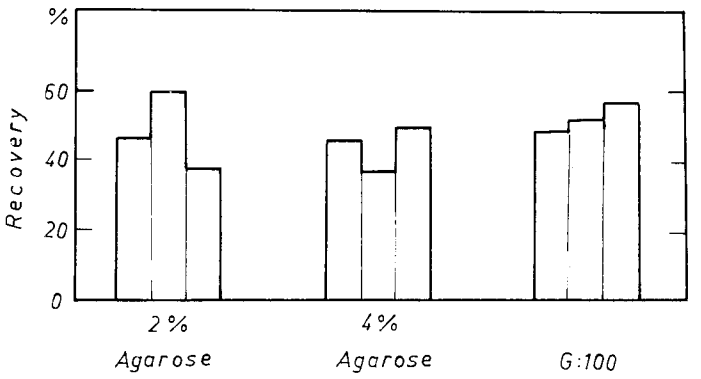

Fig. 11. The percentage of proteins recovered from 3 different separations of dental plaque material on 2 and 4 per cent agarose and Sephadex G-100 superfine.

When the elution flow rate was very low and the fractions collected were more than eighty, it took too long time to separate the enzymes and it was not possible to determine the enzymatic activity; however, by the use of this special gel (G-200 superfine) the proteins could be separated more effectively. The ratio of proteins to nucleic acids showed no differences between the two peaks in material obtained from one patient or from pooled material (Fig. 2 and 10). However, the amount of protein in each peak varied among the different samples, for example: the experiment shown in figure 10 revealed more protein in the second peak than in the corresponding peak shown in figure 2.

When 2 per cent and 4 per cent agarose was used the high molecular protein peak still was obtained (Fig. 5 and 6, respectively) coinciding with the enzymatic activity. These results suggest that Protease I is associated with high molecular elements of a molecular weight over 10^6 and could not be separated from these by the methods employed.

The parallel separation on different gels gave the main protein and protease peaks obtained previously (Fig. 6—8). However, the enzyme activity and protein concentration recovered after separation on the different media differed somewhat (Fig. 6, 7, 8 and 11). This difference may be due to some form of interac-

tion between the gels and proteins or protease enzymes in the dental plaque material or possible enzyme inhibitors in the gel material. The dental plaque supernatant added to the top of the gel may have contained considerable particulate material which could influence the recovery of proteins and proteases. This material will be held back to some degree in the layer of Sephadex G-25 at the top of the gel. The low molecular protein peak came very late in the separation, which may depend on presence of aromatic amino acids and the shape as well as the size of the molecules (Fig. 1—8).

Some of the experiments also showed a third enzyme (Protease III) peak active against both hemoglobin and gelatin (Fig. 5 c and d). This peak was apparent also when G-100 superfine was used (Fig. 7 c, 8 b and 10). However, this enzyme fraction was separated differently in relation to the low molecular protein peak. In the experiment shown in figure 7 c Protease III was eluted before the low molecular peak but in the experiment shown in figure 8 b and 10 it was coincident with the low molecular peak. The enzyme activity against BAEE coincided with the hemoglobinolytic activity Protease II (Fig. 10) was twice as active against BAEE as Protease I.

The nature of the association of the first protease with macromolecular particles remains to be determined.

Further investigation on these enzymes are in progress.

This work has been supported by the Swedish Medical Research Counlci Project No 24 x-60-02).

SUMMARY

Two main protein fractions have been separated from the supernatant of dental plaque material after low speed centrifugation with the aid of gel filtration through Sephadex and agarose columns. The proteins in one of these fractions was of high molecular weight and the other of low molecular weight. Two different proteinases were always obtained and sometimes a third enzyme fraction also appeared. The enzyme activity in the first fraction could not be separated from the high molecular prote-

ins. The enzyme in the second fraction was obtained in a rather pure form and the third enzyme fraction came close to the low molecular proteins.

RÉSUMÉ

ACTIVITÉ PROTÉOLYTIQUE DE LA PLAQUE DENTAIRE

VII. SEPARATION DE PROTÉINASES DE LA PLAQUE DENTAIRE PAR FILTRATION SUR GEL SEPHADEX ET COLONNES DE GÉLOSE

Le surnageant de matériel de plaques dentaires, obtenu par centrifugation à 2.500 g a été séparé par filtration sur gel Sephadex et gélase en deux fractions protéiniques essentielles.

L'une de ces fractions comprenait des protéines de poids moléculaire élévé et l'autre des protéines de poids moléculaire bas. En outre on a obtenu régulièrement deux protéinases différentes et plus occasionellement une troisième fraction enzymatique.

En ce qui concerne la première fraction, et dans les conditions expérimentales données, l'activité enzymatique et la substance protéinique de poids moléculaire élevé se montraient inséparables, contrairement à l'enzyme de la deuxième fraction qui a été obtenue à l'état très pur. La troisième fraction enzymatique accompagnait les protéines de poids moléculaire bas.

ZUSAMMENFASSUNG

PROTEOLYTISCHE AKTIVITÄT IN ZAHNBELAGMATERIAL

Die Supernatfraktion von Zahnbelagmaterial, erhalten nach Zentrifugieren der entsprechenden Suspension bei 2500 G, wurde mittels Gelfiltration durch Sephadex- und Agarosekolonnen separiert in zwei hauptsächlich proteine Fraktionen.

Die Proteine der einen Fraktion bestanden aus hochmolekulärem, die der anderen aus niedermolekulärem Material. Zwei verschiedene Proteasen wurden regelmässig erhalten, eine dritte Enzymfraktion gelegentlich.

Die enzymatische Aktivität der ersten Fraktion war unter den vorliegenden Versuchsbedingungen nicht von dem hochmolekulären Proteinanteil zu trennen. Das Enzym der zweiten Fraktion hingegen wurde in reiner Form erhalten. Die dritte Enzymfraktion folgte dicht auf die niedermolekulären Proteine.

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