Characterization of trypsin-like enzymes from human saliva isolated by use of affinity chromatography

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Paraffin-stimulated whole mixed saliva was collected from 6 individuals and pooled. Three trypsin-like enzymes from saliva supernatant and two from the saliva sediment were isolated by use of affinity chromatography on a soybean trypsin inhibitor Sepharose 4 B column. The isoelectric points for these enzymes were pI 8.0, 6.8 and 6.4 from the supernatant and 6.4 and 6.2 from the sediment. The enzymes were characterized with respect to pH-optimum, pH-stability, temperature stability, substrate specificity and influence of metal ions and inhibitors. The Michaelis constants were determined for these enzymes on the substrates BAEE and TAME.

Key-words: Biochemistry; physiology.

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Human saliva contains peptidases which are present in the supernatant as well as in the sediment after centrifugation at 10,000 g (*Söder*, 1972, *Lindqvist et al.*, 1974). Peptidase «bound» to the saliva sediment could be solubilized by use of the detergent sodium deoxycholate (DOC) (*Lindqvist et al.*, 1974). Gel filtration of DOC-treated saliva revealed the presence of different peptidases in saliva (*Modéer*, 1974).

Rao & Nadler (1972) pointed out that human saliva supernatant contained trypsin-like activity. This activity also has been shown by *Modéer* (1974), who found trypsin-like peptidases in human saliva supernatant as well as in the sediment.

Trypsin-like enzymes from rat and mouse submandibular glands have been extensively studied by *Riekkinen, Ekfors & Hopsu* 1966, *Riekkinen et al.* 1967. *Ekfors, Malmiharju & Hopsu-Havu*, 1972. The properties of these enzymes from the mouse and rat closely resemble each other (*Ekfors & Hopsu-Havu*, 1972).

Cuatrecasas, Wilchek & Anfinsen (1968) succeeded to prepare two sepharose derivates which had a specific affinity for chymotrypsin and carboxypeptidase A. By coupling soybean trypsin inhibitor to an insoluble matrix a biospecific adsorption of trypsin and chymotrypsin was achieved (*Porath & Sundberg*, 1970). This technique has also been used to separate bovine α- and β-trypsin (*Jameson & Elmore*, 1974).

This communication describes the isolation of trypsin like enzymes from human saliva by

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use of affinity chromatography and the characteristics of these enzymes.

MATERIAL AND METHODS

Saliva

Paraffin-stimulated whole saliva was collected from each of 3 men and 3 women (25–35 years old). The subjects had to rinse their mouths with distilled water before collection procedure, 3–4 hours after oral hygiene. The saliva samples were pooled (610 ml) and centrifuged at 10,000 g for 15 min at 4 °C. The sediment was washed twice with 0.05 M tris-HCl buffer, pH 8.1 contaning 0.5 M NaCl and centrifuged at 10,000 g. The supernatant fluid and the wash supernatants were pooled (900 ml) and called e_1. The sediment was incubated with sodium deoxycholate (DOC) 0.4% (w/v) in 0.05 M tris-HCl buffer, pH 8.1 containing 0.5 M NaCl for 3 hours at 25 °C to release the peptidases according to the method of *Lindqvist et al.*, (1974). The suspension was centrifuged at 10,000 g for 15 min at 4 °C. The sediment was washed twice with the buffer mentioned above and recentrifuged. The supernatants were pooled (610 ml) and called e_2. Fractions e_1 and e_2 were concentrated to 100 ml by amicon diafilter with H1DP 10 filter, (Amicon, Oosterhaut, Holland) and dialysed against 0.05 M tris-HCl buffer, 0.5 M NaCl pH 8.1 before affinity chromatography.

Enzyme assay

Peptidase activity was determined on following substrates: gelatin, (USP gran Fisher S-C Co., N. J., USA) (*Hultin*, 1946, 1948), α-N-benzoyl-L-arginine ethyl ester-HCl (BAEE) (Sigma Chemical Company, St. Louis, Miss., USA) (*Kétzdy, Lorand & Miller*, 1965), p-tosyl-L-arginine methyl ester-HCl (TAME) (Nutritional Biochemical Corporation, Cleveland, Ohio, USA) (*Hummel*, 1959) L-lysine-p-nitroanilide dihydrobromide (LPA), N-benzoyl-L-arginine-p-nitroanilide (BAPA) (Nutritional Biochemical Corporation) (*Erlanger, Kokowsky & Cohe*, 1961) and N-acetyl-L-tyrosine ethyl ester-HCl (ATEE) (Sigma) (*Patat & Hirsch*, 1966). The assay systems for all these substrates except ATEE and BABA for the estimation of peptidase activity in human saliva have been described previously (*Lindqvist et al.*, 1974).

Preparation of the soy bean trypsin inhibitor Sepharose gel (STI-Sepharose)

The gel was prepared according to the instruction from the manufacturer. Soybean trypsin inhibitor (Sigma) (2 mg/ml) was dissolved in 0.1 M $NaHCO_3$ buffer containing 0.5 NaCl pH 9.0 and was mixed with cyanobromide activated Sepharose 4 B (Pharmacia Fine Chemicals, Uppsala, Sweden) (10 mg soybean trypsin inhibitor/1 g Sepharose) for 2 hours at 25 °C. Unbound inhibitor was washed away with the coupling buffer and to block any remaining active groups the gel was mixed with 1.0 M ethanolamine (5 ml/g gel). Non covalentlently bound material was removed by washing three times, each with 0.1 M acetate buffer containing 1.0 M NaCl pH 4.0 and 0.1 M borate buffer containing 1.0 M NaCl pH 9.0.

Purification procedure

The affinity chromatography was used according to a method of *Porath & Sundberg*, (1970). The concentrated fractions e_1 and e_2 were introduced into the STI-Sepharose column, equilibrated with 0.05 M tris-HCl, 0.5 M NaCl buffer pH 8.1. The column was washed with 0.05 M tris-HCl, 0.5 M NaCl buffer pH 8.1 and 0.05 M tris-HCl buffer, containing 0.1 M NaCl pH 8.1 until the effluent was free from UV adsorbing material. Desorption was effected by 0.05 M glycine-HCl buffer pH 3.6 containing 0.1 M NaCl and 0.02 M $CaCl_2$. The effluent was continuously monitored by UV-adsorption at 254 nm. The adsorption at 280 and 260 nm was measured on all fractions with a Zeiss spectrophotometer PMQ II and the protein content was calculated. Before electrofocusing the material were desalted.

Isoelectric focusing

Isoelectric focusing according to *Vesterberg & Svensson* (1966) was carried out in an Ampholine column 8100 (LKB, Stockholm, Sweden) at 12–14 °C for 64 hours. The pH of the fractions was determined with a pH-meter type PHM 28 (Radiometer, Copenhagen, Denmark). Other data are given in the figure legends.

Gel electrofocusing was performed using cylindrical colums of 85 mm (*Wrigley*, 1971). The following composition of the gel was used: 5.5% (w/v) acrylamide, 0.15% (w/v) methylenebisacrylamide (bis) (Bio-Rad laboratories, Richmond, California, USA) 1.6% (v/v) ampholine pH 5–8, 0.4% (v/v) ampholine pH 3–10 (LKB) and 2.0 μg/ml riboflavin. The polymerization was carried out in fluorescence light for two hours. The samples, 0.20 ml, in 10% (w/v) sucrose were applied to the columns followed by 0.5 ml 5% (w/v) sucrose in 2.0% (v/v) ampholine pH 3–10. As anode solution 0.2% (v/v) H_2SO_4 was used and as cathode solution 0.4% (v/v) ethylenediamine, was used. The voltage was gradually increased up to a maximum of 400 V maintaining a maximum current of 3 mA per column for two hours at 4 °C. After electrofocusing the pH gradient in the gel was determined by cutting the gel into 15 equal sections and the pH of a 1.0 ml extract in distilled water from each piece was measured. The enzyme staining procedure according to a method of *Fujimoto et al.* (1972) was modified. The following concentrations of chemicals were used: 1.35 mg/ml BAEE, 33 μg/ml ADH, 0.67 mg/ml NAD, 0.2 mg/ml NBT and 6.7 μg/ml PMS in 0.1 M pyrophosphate-glycine buffer pH 9.0. The gels were incubated at 25°C for 12 hours. After the incubation time the gels were washed with with 5.0% acetic acid.

pH-optimum and pH-stability

The effect of pH on the enzymatic hydrolysis of BAEE was determined in the range of pH 5–9 using an acetate-tris-glycine buffer (0.2 M each). The substrate at the appropriate pH was used as a blank, distilled water being added instead of enzyme. To study the pH stability, the enzymatic hydrolysis was determined in the range of pH 2.5–9.5 using a glycine-acetate-tris-glycine buffer (0.2 M each).

Inhibitors and metal ions

The influence of following substances was tested on the peptidases: ethylenediaminetetraacetic acid disodiumsalt (EDTA), L+ cysteiniumchlorid, acetic acid anhydride (Merck A. G.), iodobenzoic acid, (Hopkin and Williams, Ltd. Chadwell Heath, Essex, England) p-amino-benzamidine, lima bean trypsin inhibitor, (LTI), ovomucoid trypsin inhibitor (OTI), p-chlormercuribenzoic acid (PCMB) N-tosyl-L-phenylalanine chloro-methyl ketone (TPCK) and N-p-tosyl-L-lysine chloromethyl ketone (TLCK) (Sigma). Lima bean trypsin inhibitor and ovomucoid trypsin inhibitor were used at a final concentration of $2 \cdot 10^{-4}$, $1.0 \cdot 10^{-3}$ and $5 \cdot 10^{-3}$ (w/v) and the other substances were used at final concentrations of 10^{-5}, $2 \cdot 10^{-4}$ and 10^{-3} M. The effect of the following metal ions was tested on the enzyme activity at final concentrations of $4 \cdot 10^{-5}$, $2 \cdot 10^{-4}$ and 10^{-3} M: Ca^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+} and Cu^{2+} (added as chlorides).

Kinetic determinations

Lineweaver-Burk plots of initial rates of hydrolysis of BAEE and TAME at different substrate concentrations was determined at 25 °C. The Michaelis-Menten constant (K_m) was calculated from these values.

RESULTS

Isolation

A typical elution pattern obtained after affinity chromatography on a STI-Sepharose column of the concentrated fraction e_1 and e_2 is shown in Fig. 1. At the point marked with an arrow in the figure the eluant buffer was changed abruptly to 0.05 M glycine-HCl, 0.1 M NaCl, 0.02 M $CaCl_2$ pH 3.6. The peptidase activity measured as hydrolysis of BAEE appeared in tubes numbered 29–41 for e_1 (Fig. 1 A) and in tubes numbered 27–37 for e_2 (Fig. 1 B). These tubes were pooled and called e_{11} and e_{21} respectively. Of the applied material, fraction e_{11} contained 10% of the BAEE activity but only 0.1% of the protein content. Fraction e_{21} contained 17% of the BAEE activity, 54% of the gelatinolytic activity and 0.3% of

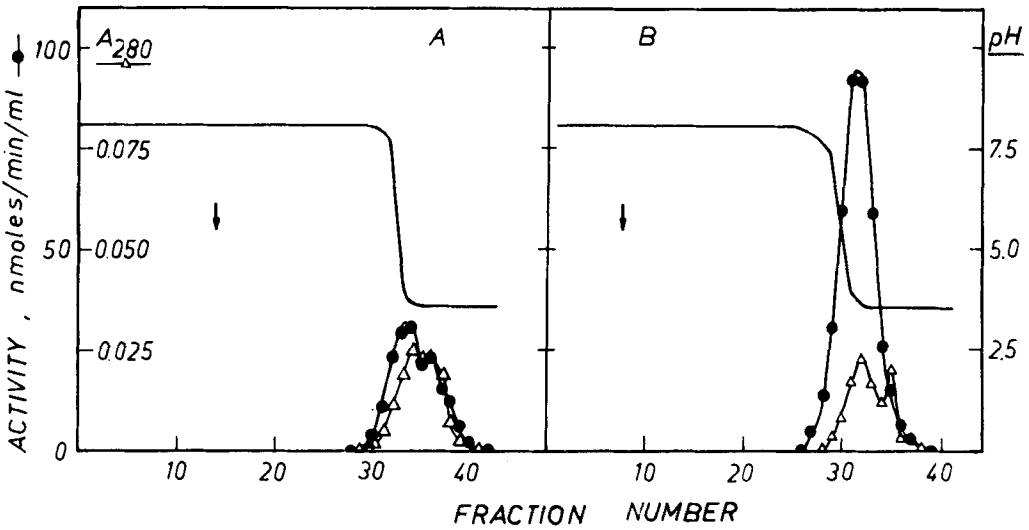

Fig. 1. Affinity chromatography of human saliva (610 ml). The saliva was centrifuged and the supernatant was concentrated to 100 ml (e_1). The sediment was treated with DOC, centrifuged and concentrated to 100 ml (e_2). Fraction e_1 (A) and e_2 (B) were separately applied on a 1.5 x 3.0 cm STI-Sepharose 4 B column. Eluant was 0.05 M glycine-HCl, 0.1 M NaCl, 0.02 M $CaCl_2$ buffer pH 3.6 marked with an arrow in the figure. Flow rate was 15 ml/h. Fraction volume 3.0 ml. The proteolytic activity was assayed on BAEE at pH 8.2 –●–, A_{280} nm = –△–, pH of the effluent = ——.

protein content of the applied material (Table I). The specific activity (U/A_{280}) for the enzymes in e_{21} was 5 times higher than the corresponding specific activity for the enzymes in e_{11} (Table I). The trypsin like enzymes in e_{11} were 85-fold purified and the corresponding purification factor for the enzymes in e_{21} was 65, when the enzymes were assayed on BAEE. The corresponding purification factor was 188 and 210 respectively when gelatin was used as substrate (Table I).

Isoelectric focusing and gelelectrofocusing

The peptidases of e_{11} was separated by isoelectric focusing (Fig. 2 A) and fraction e_{21} by gel electrofocusing (Fig. 2 B). Three different peaks were found after isoelectrofocusing of e_{11}. The main peak was found at pH 6.8 and minor peaks were found at pH 8.0 and 6.4 (Fig. 2 A).

Gel electrofocusing of e_{21} revealed two distinct bands at pH 6.4 and 6.2. Bands were also visible at pH 7.5, 6.0 and 5.8 (Fig. 2 B).

The effect of storing

The peptidases of e_{11} and e_{21} were kept at 4 °C and – 18 °C for different periods of time. The residual activity towards BAEE was measured (Fig. 3). The enzymes in e_{11} lost around 33% of their activity after one day at 4 °C and after 12 days 52% of the activity remained. At – 18 °C the enzymes in e_{11} lost 68% of their activity.

The enzyme activity of e_{21} decreased slowly to 76% of the initial value after 12 days at 4 °C. Storing at – 18 °C resulted in a 50% loss of enzyme activity.

pH optimum

The influence of pH on the peptidase activity of fraction e_{11} and e_{21} is shown in Fig. 4. The optimum enzyme activity on BAEE and TAME of fractions e_{11} and e_{21} was between pH 8.0–8.3. The pH optimum of the gelatinolytic activity for e_{11} was pH 8.0 and for e_{21} pH 8.5.

pH stability

The effect of pH on the stability of e_{11} and e_{21} enzymes is shown in Fig. 5. The enzymes of e_{21} were found to be stable from pH 3.5 to 8.5. Only small decrease of activity was noticed when the enzymes were exposed to pH lower than pH 3.5 or higher than pH 8.5. The enzy-

Table I. *Purification of trypsin like enzymes from human saliva (610 ml). The enzyme activity was determined on gelatin and BAEE at pH 8.5 and pH 8.2 respectively. The gelatinolytic activity was expressed in Hultin Units (H.U.). One unit (U) = 1 nmole of BAEE converted per ml and minute.*

Fraction	ml	A_{280} nm	Enzyme activity H.U./ml	U/ml	H.U./A_{280}	U/A_{280}	Yield % Gelatin	BAEE
e_1 (saliva supernatant)	100	4.6	25	53	5.5	11	100	100
e_{10} (non-adsorbed fraction)	100	4.4	18	43	4.3	10	71	80
e_{11} (eluted fractions)	21	0.028	29	27	1053	965	24	10
e_2 (supernatant of DOC-treated sediment)	100	1.3	51	91	39	70	100	100
e_{20} (non-adsorbed fraction)	100	1.1	23	71	21	65	46	78
e_{21} (eluted fractions)	21	0.016	132	75	8250	4700	54	17

mes of e_{21} were stable between pH 6.0–8.5. There was a slow decrease of activity at pH values below pH 6.0.

Temperature stability

The temperature stability of the enzymes of e_{11} and e_{21} is shown in Fig. 6.

After 30 min incubation at 25 °C and 60 °C, 79% and 43%, respectively of the original activity of e_{11} remained. After 30 min incubation at 60 °C, 96% of the enzyme activity of e_{21} remained.

Substrate specificity

Table II shows the relative hydrolysis rates of different substrates by purified peptidases in e_{11} and e_{21} measured as nmoles/ml/min and the Michaelis constant on BAEE and TAME. The enzymes of e_{11} and e_{21} hydrolysed TAME faster than BAEE, were inactive against LPA and ATEE, and hydrolyzed BAPA slowly. The K_m values for the enzymes of fraction e_{11} and e_{21} against BAEE at pH 8.2 was $5.0 \cdot 10^{-5}$ and $6.0 \cdot 10^{-5}$ respectively. The corresponding K_m value towards TAME was $3.7 \cdot 10^{-4}$ and $3.0 \cdot 10^{-4}$ (Table II) for e_{11} and e_{21}, respectively.

Influence of metal ions

The effect of metal ions on the peptidases in e_{11} and e_{21} (Table III) disclosed that Ca^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+} and Cu^{2+} had no significant influence on the enzyme activity. However, cobolt at a concentration of $2 \cdot 10^{-4}$ M resulted in an inhibition of the activity of both e_{11} and e_{21} by around 65%.

Influence of various inhibitors

The effect of inhibitors on the enzyme activity of e_{11} and e_{21} is shown in Table IV. Benzamidine at a concentration of $2 \cdot 10^{-4}$ M inhibited the enzyme activity of e_{11} and e_{21} so that 41% and 62% respectively of the activity remained. EDTA at all concentrations used inhibited the enzyme activity of both e_{11} and e_{21} by around 20%. Incubation of the enzymes with 10^{-3} M

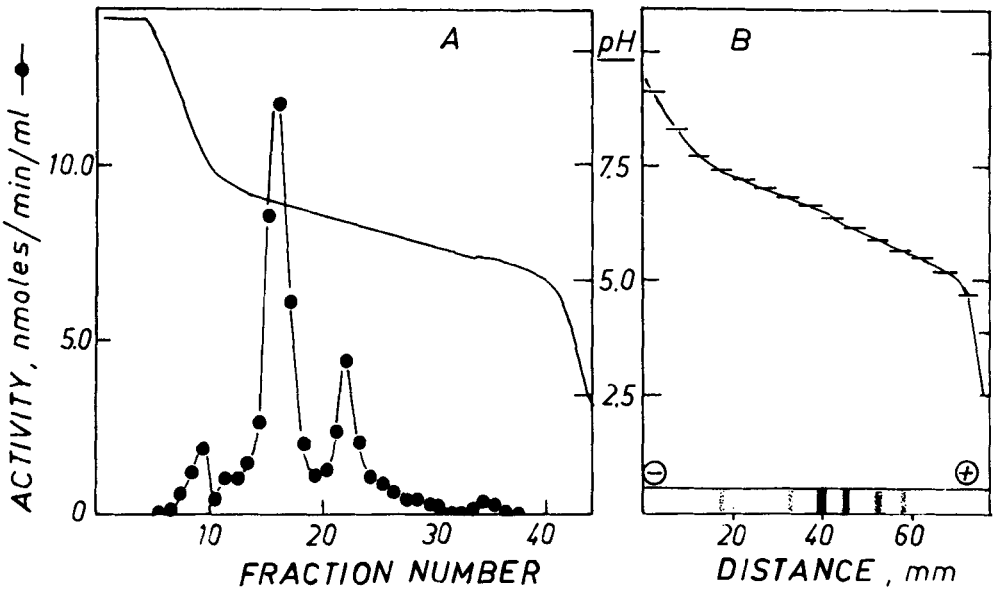

Fig. 2A. Isoelectric focusing of fraction e_{11} on a 110 ml column. Range of the carrier ampholine (50% pH 3–10 and 50% pH 5–7). Time for isoelectric focusing: 64 h, voltage 500 V, current ~ 1.0 mA. The proteolytic activity assayed on BAEE at pH 8.2 = –●–, pH of the effluent = ——.

Fig. 2B. Gel electrofocusing of fraction e_{21} on cylindrical columns of 85 mm for 2 h at 4 °C. Range of carrier ampholine (0.4% pH 3–10 and 1.6% pH 5–8). Voltage ~ 400 V, current ~ 3 mA per column. pH of the sliced pieces = —. The proteolytic activity was determined at pH 9.0 with BAEE as substrate. The enzyme activity in the sliced sections is marked as shaded areas at the bottom of the figure.

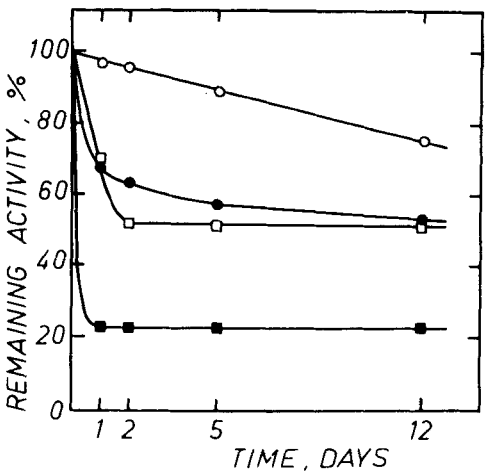

Fig. 3. The effect of storing the enzymes of fractions e_{11} and e_{21} at 4 °C and –18 °C. The remaining enzymatic activity was assayed on BAEE at pH 8.2. o–o = fraction e_{11} at 4 °C. ●–● = fraction e_{21} at 4 °C. ■–■ = fraction e_{11} at –18 °C. □–□ = fraction e_{21} at –18 °C.

TLCK resulted in a stronger inhibition of the activity of e_{11} than e_{21}. After treatment of fraction e_{11} and e_{21} with $2 \cdot 10^{-4}$ M about 65% of the activity remained. The ovomucoid trypsin inhibitor and TPCK had no influence on the enzyme activity of e_{11} or e_{21}.

DISCUSSION

Attempts to purify proteolytic enzymes from human saliva as well as from salivary glands of animals have met with only partial success when conventional separatory procedures have been used (*Mäkinen*, 1966), *Ekfors, Malmiharju & Hopsu-Havu*, 1972, *Söder*, 1972, *Modéer*, 1974). In recent years, affinity chromatography has been exploited in the purification of enzymes (*Cuatrecasas, Wilchek & Anfinsen*, 1968, *Porath & Sundberg*, 1970,

Table II. *The substrate specificity of e_{11} and e_{21} enzymes.*

Substrates	Rel. enzyme conc.	Temp.	Rel. activity		Km mM	
			e_{11}	e_{21}	e_{11}	e_{21}
TAME	1	25	100	100	0,37	0,30
BAEE	1	25	84	81	0,05	0,06
BABA	2.5	37	4	3	–	–
ATEE	1	25	0	0	–	–
LPA	2.5	37	0	0	–	–

Feinstein & Gertler, 1973, *Lievaart & Stevenson*, 1974). This technique has been used in this study by coupling soybean trypsin inhibitor which is a substrate analogue inhibitor (*Modéer*, 1974) to cyanogen bromide activated Sepharose 4 B, achieving biospecific adsorption of the trypsin-like enzyme to the matrix. The coupled enzyme could be eluted from the column either by changing the ion strength or pH of the eluant (*Cuatrecasas & Anfinsen*, 1971 a, b, *Friedberg*, 1971). *Porath & Sundberg* (1970) found that trypsin and chymotrypsin were released at pH 3.3 and pH 4.5, respectively, from the STI-Sepharose.

The material applied to the column must be supplemented with 0.5 M NaCl to avoid non specific adsorption of the salivary proteins to the column during the chromatography.

Rao & Nadler (1972) found that human saliva contained enzymatic activity which could hydrolyse BAEE and was inhibited by soybean trypsin inhibitor. These enzymes found in human saliva could be isolated by use of affinity chromatography (Fig. 1).

The purified enzymes (e_{11} and e_{21} in Fig. 1) showed similarities to trypsin because of their capacity to hydrolyze BAEE, TAME and BAPA (Table II) and their inhibition by TLCK (Table IV). In contrast to trypsin, these enzymes did not hydrolyse the substrate LPA (Table II) and were unaffected by TPCK (Table IV). The pH optimum of these enzymes were close to that of bovine trypsin when BAEE and TAME were used as substrates (Fig. 4) (*Walsh & Wilcox*, 1970). Isoelectric focusing of the trypsin-like enzymes in e_{11} revealed three different peaks (Fig. 2 A). The main peak was found at pH 6.8. This enzyme may represent the same proteolytic enzyme found by *Söder* (1972) after isoelectrofocusing of dental plaque material since it had the same pI

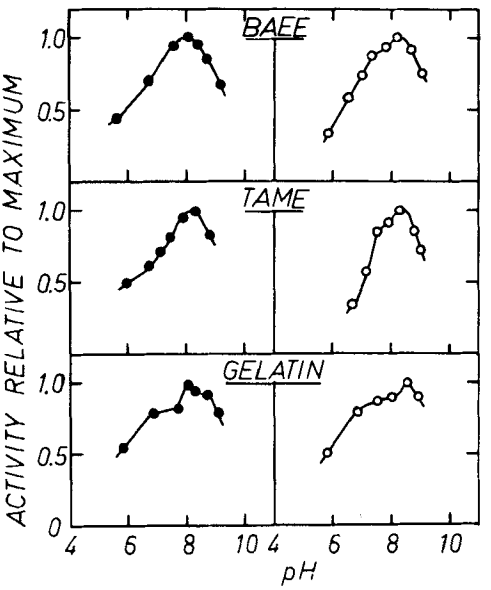

Fig. 4. The influence of pH on the hydrolysis of BAEE, TAME and gelatin by the enzymes of fractions e_{11} (closed symbols) and e_{21} (open symbols).

and was unaffected by addition of Ca^{2+}. The molecular weight of this enzyme was calculated to be around 24,000.

Gel electrofocusing of trypsin like enzymes of type e_{21} revealed two distinct bands at pH 6.4 and 6.2 (Fig. 2 B). These enzymes were more sensitive than the enzymes of e_{11} to pH values lower than pH 6.0. Therefore the enzymes of e_{21} were separated by gel electrofocusing which have a shorter separation time with smaller loss of enzymatic activity. It has not been possible, so far, to characterize the isolated enzymes after isoelectric focusing as the yields were too low.

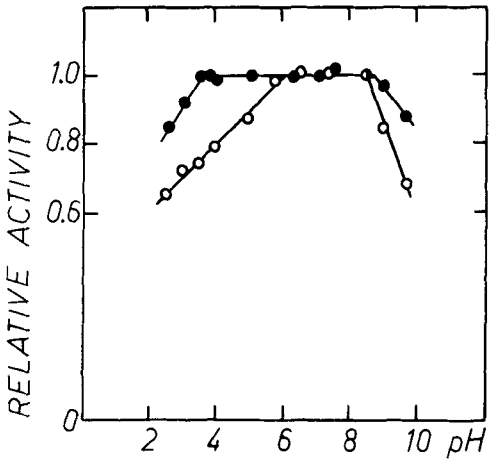

Fig. 5. The effect of pH on the stability of the enzymes of fractions e_{11} and e_{21}. The enzyme solutions were incubated with buffers of different pH for 30 min at 25 °C and the activity remaining was determined on BAEE at pH 8.2. Closed symbols = The effect of pH on the stability of the enzymes in fraction e_{11}. Open symbols = The effect of pH on the stability of the enzymes in fraction e_{21}.

Table III. *Influence of various metal ions on the enzyme of e_{11} and e_{21}. Activities were determined on BAEE at pH 8.2.*

Metal ions	Final conc.	Enzyme activity as % of control Fraction e_{11}	Fraction e_{21}
Ca^{2+}	$4 \cdot 10^{-5}$ M	100	98
	$2 \cdot 10^{-4}$ M	109	100
	$1 \cdot 10^{-3}$ M	107	99
Mn^{2+}	$4 \cdot 10^{-5}$ M	100	100
	$2 \cdot 10^{-4}$ M	84	91
	$1 \cdot 10^{-3}$ M	74	90
Mg^{2+}	$4 \cdot 10^{-5}$ M	98	99
	$2 \cdot 10^{-4}$ M	102	95
	$1 \cdot 10^{-3}$ M	97	94
Ni^{2+}	$4 \cdot 10^{-5}$ M	100	97
	$2 \cdot 10^{-4}$ M	106	90
	$1 \cdot 10^{-3}$ M	105	89
Co^{2+}	$4 \cdot 10^{-5}$ M	99	90
	$2 \cdot 10^{-4}$ M	34	37
Cu^{2+}	$4 \cdot 10^{-5}$ M	105	90
	$2 \cdot 10^{-4}$ M	85	87

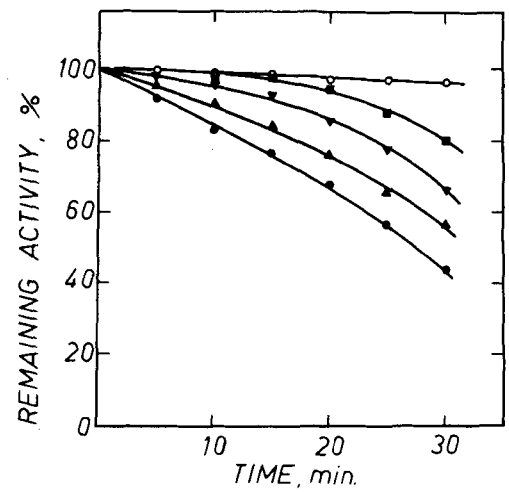

Fig. 6. The effect of temperature on the stability of the enzymes of e_{11} and e_{21}. The enzymes were incubated at different temperatures up to 30 min at pH 8.0 and the activity remaining was determined on BAEE at pH 8.2. ■–■ = fraction e_{11} at 25 °C. ▼–▼ = fraction e_{11} at 40 °C. ▲–▲ = fraction e_{11} at 50 °C. ●–● = fraction e_{11} at 60 °C. o–o = fraction e_{21} at 60 °C.

Studies on temperature sensitivity suggested that these enzymes were sensitive to freezing or thawing (Fig. 3). The trypsin-like enzymes were also sensitive to storing at 4 °C, especially e_{11}, which is in agreement with the finding of *Rao & Nadler,* (1972).

Affinity chromatography offers the possibility of isolating a specific group of enzymes by a one step procedure from a large volume of biological material. However the isolated trypsin-like enzymes are only a part of the total peptidase activity in human saliva (Table I). By use of other substrate analogs it may be possible to isolate the different proteolytic enzymes in the nonadsorbed fraction.

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Table IV. *Influence of various inhibitors on the enzyme activity of e_{11} and e_{21}. The inhibitors TLCK, TPCK and PCMB were preincubated with the enzymes for 30 min. For abbreviations, see Material and Methods. The rest of inhibitors were preincubated with the enzyme for 5 min. Activities were determined on BAEE at pH 8.2.*

Substances	Final conc.	Enzyme activity as % of control	
		e_{11}	e_{21}
Lima bean trypsin inhibitor	$5 \cdot 10^{-3}$ %	100	100
	$5 \cdot 10^{-2}$ %	100	94
	$1 \cdot 10^{-2}$ %	100	88
Ovomucoid trypsin inhibitor	$5 \cdot 10^{-3}$ %	104	100
	$5 \cdot 10^{-2}$ %	104	100
	$1 \cdot 10^{-2}$ %	104	100
EDTA	$4 \cdot 10^{-5}$ M	80	85
	$2 \cdot 10^{-4}$ M	80	82
	$1 \cdot 10^{-3}$ M	78	81
Acetic acid anhydride	$4 \cdot 10^{-5}$ M	81	88
	$2 \cdot 10^{-4}$ M	79	85
	$1 \cdot 10^{-3}$ M	79	83
L-cysteine	$4 \cdot 10^{-5}$ M	72	85
	$2 \cdot 10^{-4}$ M	70	80
	$1 \cdot 10^{-3}$ M	65	67
p-aminobenzamidine	$4 \cdot 10^{-5}$ M	64	93
	$2 \cdot 10^{-4}$ M	41	62
Iodobenzoic acid	$4 \cdot 10^{-5}$ M	54	57
	$2 \cdot 10^{-4}$ M	53	19
PCMB	$4 \cdot 10^{-5}$ M	100	90
	$2 \cdot 10^{-4}$ M	63	65
TLCK	$4 \cdot 10^{-5}$ M	94	100
	$2 \cdot 10^{-4}$ M	63	84
	$1 \cdot 10^{-3}$ M	47	61
TPCK	$4 \cdot 10^{-5}$ M	100	100
	$2 \cdot 10^{-4}$ M	95	93

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