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Isolation of a Chymotrypsinlike Enzyme from *Treponema denticola*

VELI-JUKKA UITTO,[1]* DANIEL GRENIER,[2] E. C. S. CHAN,[3] AND BARRY C. McBRIDE[1,2]

Department of Oral Biology[1] and Department of Microbiology,[2] University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z7, and Department of Microbiology and Immunology,[3] McGill University, Montreal, Quebec, Canada H3A 2B4

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A chymotrypsinlike protease with an M_r of 95,000 was extracted from *Treponema denticola* ATCC 35405 and was partially purified by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteolytic activity was detected in an electrophoretogram containing polyacrylamide that was conjugated to bovine serum albumin. A single band of activity was detected when the *T. denticola* extract was solubilized and electrophoresed in the presence of sodium dodecyl sulfate. No activity was found in extracts of *Treponema vincentii*. The enzyme hydrolyzed transferrin, fibrinogen, α_1-antitrypsin, immunoglobulin A, immunoglobulin G, gelatin, bovine serum albumin, and a synthetic peptide containing phenylalanine. It did not degrade collagen or synthetic substrates containing arginine or proline. For the hydrolysis of azocoll, the pH optimum of the enzyme was 7.5. Heating at temperatures above 50°C destroyed the activity. Reducing agents and the chelators EDTA and ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid increased the enzyme activity, while phenylmethylsulfonyl fluoride, L-1-tosylamide-2-phenylethyl chloromethyl ketone, sulfhydryl reagents, and human serum reduced activity. The ability of the enzyme to hydrolyze a number of humoral proteins suggests that it may be involved in spirochete invasiveness and tissue destruction.

Oral spirochetes from the genus *Treponema* form a significant segment of the subgingival bacterial flora in humans (1, 11, 12, 18). The proportion of spirochetes relative to other subgingival plaque bacteria drastically increases during periodontal diseases and can form up to 50% of the microscopically detectable flora (1, 12, 13). *Treponema denticola* is one of the spirochete species most frequently associated with diseased periodontal sites (18). The spirochetes appear to localize primarily on the loose surface layer of subgingival plaque close to the epithelium of the gingival pocket (11). It has been shown recently that oral treponemes have the ability to adhere to the epithelial cells and cause morphological damage (21, 22). It is also known that spirochetes are capable of invading connective tissue in some forms of periodontal disease (6, 10, 17, 23). Thus, these bacteria have the potential to have a direct deleterious effect on periodontal connective tissue. Suppression of fibroblast proliferation by oral spirochetes in vitro has been described elsewhere (2). Little is known about the virulence factors of the oral treponemes. Some studies have indicated that *T. denticola* produces a number of enzymes capable of degrading synthetic peptides (4, 9, 14, 15, 20). At least two of these enzymes preferentially catalyze the hydrolysis of arginine-containing substrates (4, 14, 20, 26). Two other peptidases that are prevalent in *T. denticola* have specificities towards proline (14). Activity against a phenylalanine-containing substrate has also been observed in the organism (9). These enzymes appear to have generally low activity against native proteins (14, 15, 20). Other studies have shown that the organism produces true proteases capable of degrading proteins such as basement membrane collagen (26; V.-J. Uitto, T. Laakso, and T. Salo, Oral Microbiol. Immunol., in press), elastin (15), fibrin (19), and keratin (16). We extend these studies by describing the isolation and partial purification of a chymotrypsinlike protease from *T. denticola* ATCC 35405 that has the ability to hydrolyze many functionally important serum and tissue proteins.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *T. denticola* ATCC 33520 and ATCC 35405, and *Treponema vincentii* ATCC 35580 were cultured anaerobically in liquid medium containing 12.5 mg of brain heart infusion, 10 mg of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 2.5 mg of yeast extract, 0.5 mg of sodium thioglycolate, 1 mg of L-cysteine, 0.25 mg of L-asparagine, 2 mg of glucose, 6 μg of thiamine pyrophosphate, and 2 mg of sodium bicarbonate per ml; 2% rabbit serum; and 0.2% volatile fatty acids, as described earlier (3). Five-day cultures of spirochetes were harvested by centrifugation, washed with phosphate-buffered saline (0.15 M NaCl, pH 7.5), and freeze-dried.

Extraction and localization of enzyme. Lyophilized spirochetes (3 mg) were dispersed by pipetting them into 1 ml of 0.05 M Tris hydrochloride buffer (pH 7.5). This cell suspension was shaken at 20°C for 30 min. The samples were then centrifuged at 13,000 × *g* for 10 min. The supernatant (fraction 1) was collected, and the cell pellet was sonicated in the presence of the same volume of the buffer for 45 s (30% duty cycle, output 5; Sonifier Cell Disrupter; Branson Sonic Power Co., Danbury, Conn.; in an ice bath). The soluble fraction was collected by centrifugation at 13,000 × *g* for 10 min (fraction 2). In some cases, cells were sonicated for a longer time (five intervals of 45 s). Unbroken cells and cellular debris were removed by centrifugation at 13,000 × *g* for 10 min (fraction 3). Membranes were then removed from the supernatant by centrifugation at 120,000 × *g* for 4 h (the supernatant was designated fraction 4 and the pellet was designated fraction 5).

Protein-conjugated PAGE. Transferrin, α_1-antitrypsin, fibrinogen, immunoglobulin G, or bovine serum albumin (BSA) was covalently bound to linear polyacrylamide (BDH, Poole, England) by the method of Kelleher and Juliano (7). Polyacrylamide gel electrophoresis (PAGE) in the presence

of sodium dodecyl sulfate (SDS) was carried out by the Laemmli method (8), by using 12% gels, 0.75 mm in thickness, containing the protein-polyacrylamide conjugate. Prior to casting, the protein-polyacrylamide conjugate was added to the gel to give a final protein concentration of 100 μg/ml. Before being loaded on the gel, treponeme extracts were incubated for 30 min at 37°C in the sample buffer that contained 1% SDS. After electrophoresis, the gels were gently shaken in 0.1 M Tris hydrochloride buffer (pH 7.0) containing 2% Triton X-100. This was followed by a wash in 0.1 M Tris hydrochloride buffer (pH 7.0) to remove the detergents. Gels were then incubated at 37°C for 2 h in 0.1 M Tris hydrochloride buffer (pH 7.0) supplemented with 50 mM L-cysteine to allow the enzymatic degradation of the conjugated protein substrates. Gels were then stained for proteins with Coomassie brilliant blue. After the gels were destained, the presence of proteolytic activity was visualized as a clear band against a blue background. The molecular weight standards were myosin (200,000), phospholipase *b* (97,400), BSA (68,000), ovalbumin (43,000), α-chymotrypsinogen (25,700), β-lactoglobulin (18,400), and cytochrome *c* (12,300).

Purification of protease. Lyophilized *T. denticola* ATCC 35405 cells were sonicated for 45 s in 0.05 M Tris hydrochloride buffer (pH 7.5). Unbroken cells were removed by centrifugation at 13,000 × *g* for 5 min. A sample of the extract, containing 30 mg of protein, was mixed with an equal volume of 0.125 M Tris hydrochloride buffer (pH 7.0) containing 2% SDS and 20% glycerol and was incubated at 37°C for 30 min. The sample was then loaded on a 12% SDS-polyacrylamide gel, 6 mm in thickness, and run at a constant current of 300 mA with the buffer system of Laemmli and water cooling. After electrophoresis, a narrow vertical strip was cut from a side of the gel. The strip was assessed for proteolytic activity in a casein clotting assay (5) as follows. The gel was rinsed in 2.5% Triton X-100 for 60 min and then was equilibrated with 0.3 M sodium acetate buffer (pH 5.3) for 60 min. The protease activity was detected by laying the gel strip on an agarose gel containing skim milk. After incubation at 37°C for 2 h, the enzyme activity was detected as a white band in a clear agarose gel. The section corresponding to the active enzyme was cut out of the remaining gel, and the enzyme was electroeluted (Bio-Rad Laboratories, Richmond, Calif.) at 10 mA per tube for 5 h. The eluate was dialyzed for 18 h against an excess of 0.01 M Tris hydrochloride buffer (pH 7.2) and then was lyophilized and reconstituted in 3 ml of 0.05 M Tris hydrochloride buffer (pH 7.2). The preparative gel electrophoresis and elution were repeated to obtain a single protein band by silver nitrate stain. Protein was quantitated by a Bio-Rad microassay.

Determination of substrate specificity of enzyme. Peptidase activities were assayed by incubating 5 μg of the enzyme with 1 mM chromogenic *p*-nitroanilide amino acid substrates in a final volume of 500 μl of 0.05 M Tris hydrochloride–0.2 M NaCl buffer (pH 7.8) for 18 h at 37°C. The change of optical density at 405 nm was recorded spectrophotometrically. Azocoll-, azocasein-, and rhemazo billiant blue elastin-degrading activities were assayed by incubating 3 mg of the substrate with the enzyme as described above. Optical densities of the degraded colored peptides of azocoll and rhemazo brilliant blue elastin were read at 520 and 595 nm, respectively. Degradation of azocasein was measured as the change in optical density at 366 nm after removal of undegraded protein by precipitation with 5% trichloroacetic acid. Degradation of BSA, α_1-antitrypsin, transferrin, fibrinogen, immunoglobulin G, immunoglobulin A, gelatin, and type I collagen was assayed by incubation of 35 μg of the protein with the enzyme for 12 h, analysis of the reaction products by SDS-PAGE by the Laemmli method (8), and staining of the proteins with Coomassie brilliant blue. The temperature was 37°C, but incubation with collagen was performed at 25°C to ensure that the native helical conformation of the molecule would be maintained. Collagen was prepared from chick leg tendons as described earlier (25).

TABLE 1. Hydrolysis of BAPNA, SAAPNA, and BSA by fractionated *T. denticola* ATCC 35405 cells

Fraction	Hydrolysis of:		
	BAPNA[a]	SAAPNA[a]	BSA[b]
1	27.7	24.4	+
2	4.5	34.9	+
3	0.8	11.4	+
4	2.1	0.7	−
5	1.8	25.0	+

[a] Change in optical density measured at 405 nm/h per ml.
[b] Activity was detected as a 95-kilodalton band in the BSA-conjugated SDS-PAGE assay.

Determination of pH optimum. The purified enzyme (5 μg) was incubated at 37°C with azocoll for 18 h at different pHs. The following buffers were used: 0.5 M citrate buffer (pH 4 to 6), 0.5 M Tris hydrochloride buffer (pH 7 to 9), and 0.5 M carbonate buffer (pH 10 to 11). A buffer blank was included at each pH, and its optical density value was subtracted from that of the enzyme samples.

Determination of heat stability. The enzyme was incubated in 0.05 M Tris hydrochloride buffer (pH 7.2) for 18 h at −20, 4, 25, or 37°C, and its activity was assayed. In other experiments, the enzyme was heated for 1 h at 40, 45, 50, 55, or 60°C before the proteolytic activity was assayed, with azocoll as substrate.

Studies with enzyme inhibitors. Several substances which are known to inhibit proteolytic enzymes from the serine, metallo, carboxyl, or sulfhydryl proteases were incubated with the enzyme for 30 min at 37°C, and the proteolytic activity was assayed, with azocoll as substrate.

Chemicals. The synthetic substrates, enzyme inhibitors, azocoll, azocasein, and purified proteins were obtained from Sigma Chemical Co., St. Louis, Mo. Rhemazo brilliant blue elastin was purchased from Elastin Products, St. Louis, Mo. Reagents for gel electrophoresis were obtained from Bio-Rad.

RESULTS

Extraction and purification of enzyme. *T. denticola* and *T. vincentii* were washed in a Tris hydrochloride buffer and then disrupted by sonication. The various fractions were assayed for trypsinlike and chymotrypsinlike enzymes by using the synthetic amino acid peptides. While very little enzyme activity was found in *T. vincentii*, there was strong enzyme activity against both *N*-α-benzoyl-L-arginine-*p*-nitroanilide (BAPNA) and succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine-*p*-nitroanilide (SAAPNA) in both strains of *T. denticola*. Table 1 shows that 83% of the BAPNA-degrading activity was recovered in fraction 1, the cell wash. In contrast, only 27% of the SAAPNA-degrading activity was present in this fraction. Fraction 2, the low speed centrifugation supernatant of sonicated *T. denticola* cells, contained high levels of activity against SAAPNA, but

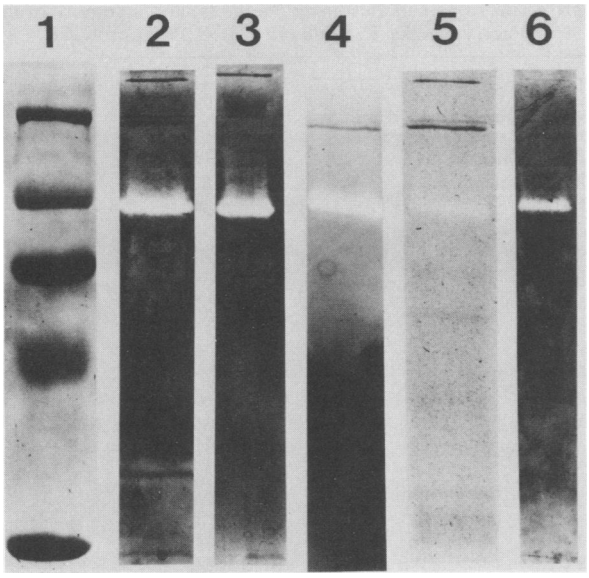

FIG. 1. Detection of proteolytic activity in *T. denticola* ATCC 35405 by protein-conjugated SDS-PAGE. *T. denticola* was sonicated in 0.05 M Tris hydrochloride buffer (pH 7.5), and the supernatant (centrifugation, 13,000 × *g*) was electrophoresed on 12% polyacrylamide gels containing the following conjugated proteins: lane 2, BSA; lane 3, α_1-antitrypsin; lane 4, transferrin; lane 5, fibrinogen; and lane 6, immunoglobulin G. The standards in lane 1 are myosin (200,000), phospholipase *b* (97,400), BSA (68,000), ovalbumin (43,000), and cytochrome *c* (12,300). Gels were stained with Coomassie brilliant blue.

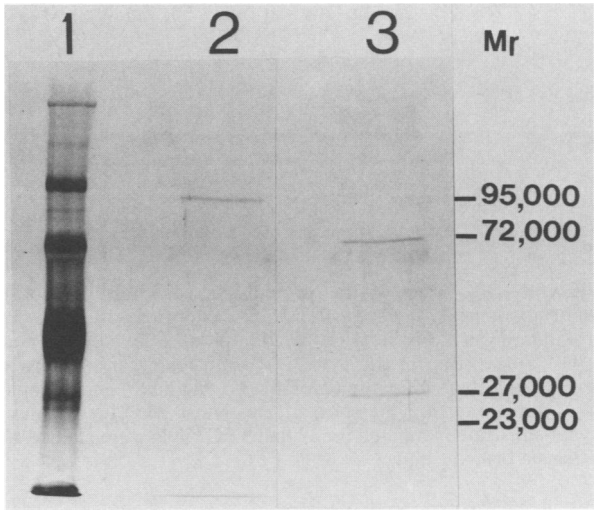

FIG. 2. 12% SDS-PAGE and silver nitrate stain of the purified chymotrypsinlike *T. denticola* ATCC 35405 protease. Lane 1, Protein standards as in Fig. 1 except that α-chymotrypsinogen (25,700) replaced cytochrome *c*; lane 2, purified protease at 37°C in the presence of SDS; lane 3, purified protease at 100°C in the presence of SDS.

none of this activity could be detected in fraction 4 (supernatant from centrifugation at 120,000 × *g*). This indicates that the enzyme was primarily located in a particulate cell fraction. In contrast, BAPNA-degrading activity was found in fraction 4 (supernatant from centrifugation at 120,000 × *g*). The SAAPNA-degrading activity in the extracts was relatively stable in the presence of 0.1% SDS. In contrast, SDS strongly inhibited the BAPNA-degrading activity. The sonic extracts of *T. denticola* ATCC 35405 were subjected to SDS-PAGE with polyacrylamide conjugated to BSA. A single proteolytic band which was shown to be active against the phenylalanine-containing substrate was detected in fractions 1, 2, 3, and 5, but no activity was detected in fraction 4. In the presence of SDS, the protein ran as a single sharp band corresponding to an apparent M_r of 95,000 (Fig. 1). The proteolytic activity could not be detected when SDS was left out of the gel and the running buffer. Similar results were obtained from *T. denticola* ATCC 35405 and ATCC 33520.

The SAAPNA-degrading enzyme from *T. denticola* ATCC 35405 was partially purified by electroelution from a preparative SDS-polyacrylamide gel. The protein content of this fraction was approximately 0.05% of the original protein that was loaded on the polyacrylamide gel, and the enzyme activity recovered was about 4% of the original. Therefore, an 80-fold purification could be obtained with this single-step procedure. SDS-PAGE and silver nitrate staining of the purified protease showed some low-molecular-weight contaminants in the preparation. When the preparative electrophoresis of the enzyme was repeated under identical conditions with the eluted fraction, a purification to near homogeneity was obtained (Fig. 2). Electrophoresis of the protein solubilized at 37°C in SDS yielded a single sharp proteolytic band that corresponded exactly with a silver nitrate-stained band having an M_r of 95,000. When the sample was heated to 100°C in SDS, three polypeptides of 72,000, 27,000, and 23,000 M_rs were obtained. The addition of β-mercaptoethanol to the SDS buffer did not alter the migration pattern of the polypeptides.

Substrate specificity of enzyme. The specificity of the *T. denticola* protease was studied with synthetic amino acid substrates. The enzyme appeared to have high specificity for phenylalanine-containing peptides. There was very little degradation of *N*-acetyl-tyrosine–*p*-nitroanilide, and there was no degradation of the other synthetic substrates tested (Table 2). The enzyme degraded azocoll and azocasein but

TABLE 2. Substrate specificity of the chymotrypsinlike *T. denticola* ATCC 35405 protease

Substrate	Enzyme activity with[a]:	
	No cysteine	25 mM cysteine
N-α-Benzoyl-L-arginine–*p*-nitroanilide	0	0
Succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine–*p*-nitroanilide	0.3	1.02
N-*p*-Tosyl-L-glycyl-L-prolyl-L-lysine–*p*-nitroanilide	0	0
N-Acetyl-L-tyrosine-*p*-nitroanilide	0	0.05
L-α-Glutamyl–*p*-nitroanilide	0	0
L-Proline–*p*-nitroanilide	0	0
L-Methionine–*p*-nitroanilide	0	0
Azocoll	0.12	0.22
Azocasein	0.25	0.33
Rhemazo brilliant blue elastin	0	0

[a] Enzyme activity is expressed as change in optical density (at 405 nm for *p*-nitroanilide substrates, at 520 nm for azocoll, at 366 nm for azocasein, and at 595 nm for rhemazo brilliant blue elastin) of the solution.

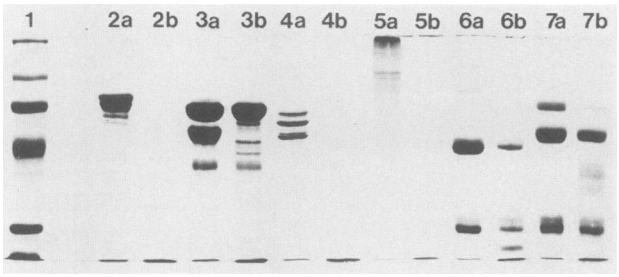

FIG. 3. SDS-PAGE of the protein cleavage products of the chymotrypsinlike *T. denticola* ATCC 35405 protease. Lane 1, Protein standards as in Fig. 1, with the addition of α-chymotrypsinogen (25,700); lane 2, transferrin; lane 3, α_1-antitrypsin; lane 4, fibrogen; lane 5, gelatin; lane 6, immunoglobulin G; and lane 7, immunoglobulin A. Lanes a, Enzyme-substrate tested at zero time; lanes b, enzyme-substrate incubated for 12 h at 37°C. Gels were stained with Coomassie brilliant blue.

not rhemazo brilliant blue elastin (Table 2). The *T. denticola* ATCC 35405 extract was also electrophoresed in gels containing immunoglobulin G, transferrin, α_1-antitrypsin, or fibrinogen conjugated to polyacrylamide. All these substrates were degraded, and in all cases the proteolysis was confined to a 95,000-M_r band (Fig. 1). The type of proteolytic cleavage produced by the protease was studied by incubating the enzyme with several proteins and analyzing the reaction products by SDS-PAGE (Fig. 3). The following observations were made. The protease degraded transferrin, fibrinogen, and gelatin into fragments which were not detectable by SDS-PAGE. The 55,000-M_r peptide of α_1-antitrypsin was partially converted to a 47,000-M_r peptide. The two other peptides of α_1-antitrypsin were not degraded. The 52,000- and 24,000-M_r peptides of immunoglobulin G were partially degraded, and a fragment of 21,000 M_r was produced. Immunoglobulin A appeared to be more resistant to the hydrolysis; however, some degradation of the 62,000- and 26,000-M_r peptides was observed. The enzyme did not degrade type I collagen.

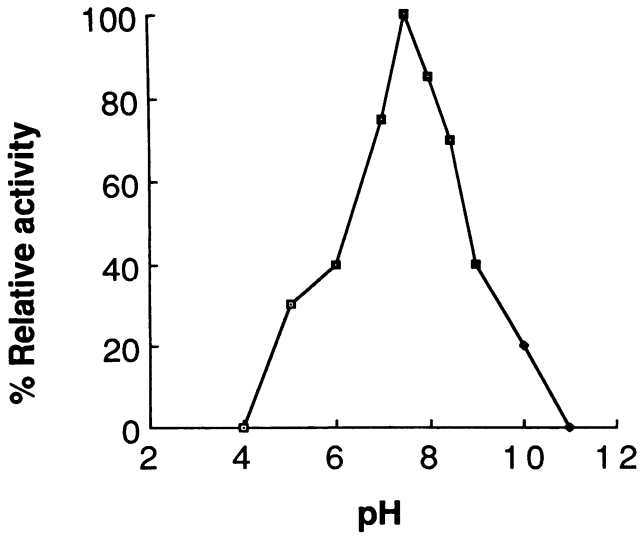

FIG. 4. pH optimum of the chymotrypsinlike *T. denticola* ATCC 35405 protease. Incubation with azocoll was performed at 37°C for 18 h. Symbols: ⊡, citrate buffer; ■, Tris hydrochloride buffer; and ◆, carbonate buffer.

TABLE 3. Effects of various compounds on the activity of the chymotrypsinlike *T. denticola* ATCC 35405 protease[a]

Expt and substance	Concn	Enzyme activity (% of control)
Expt 1		
None (control)		100
Dithiothreitol	2 mM	138
Cysteine	25 mM	166
Glutathione (oxidized)	2 mM	65
p-Chloromercuribenzoic acid	2 mM	10
$HgCl_2$	2 mM	26
Iodoacetic acid	2 mM	20
Expt 2		
None (control)		100
EDTA	10 mM	130
EGTA	10 mM	165
1,10-Phenanthroline	10 mM	60
PMSF	2 mM	11
Soybean trypsin inhibitor	10 μg/ml	81
α_1-Antitrypsin	10 μg/ml	90
TLCK	2 mM	110
TPCK	2 mM	35
Pepstatin	10 μg/ml	85
Human serum	0.5%	40

[a] The enzyme activity was measured by incubating the enzyme and the inhibitors with azocoll at 37°C for 16 h. In experiment 2, all samples were incubated in the presence of 25 mM cysteine.

pH optimum and stability of protease. The optimum pH for the enzyme activity was found to be about 7.5. More than 20% of the activity was still present at pHs of 5.0 and 10.0 (Fig. 4). The protease was found to be relatively heat stable. Storage at −20, 4, 25, and 37°C for 24 h or heating for 60 min at 50°C did not appear to change the enzyme activity. Heating at 55°C for 1 h reduced the enzyme activity by about 90%, and at 60°C the activity was totally lost.

Effects of enzyme inhibitors. The enzyme was sulfhydryl dependent, since cysteine and dithiothreitol increased the activity and since oxidized glutathione, *p*-chloromercuribenzoic acid, iodoacetic acid, and $HgCl_2$ inhibited activity (Table 3). The enzyme activity was inhibited by the serine protease inhibitors phenylmethylsulfonyl fluoride (PMSF) and L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK), each at a concentration of 2 mM, but was not inhibited by 2 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK) or 10 μg of either soybean trypsin inhibitor or α_1-antitrypsin per ml. Human serum at a concentration of 0.5% reduced the enzyme activity. Increased enzyme activity was noted in the presence of the chelators EDTA and ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA).

DISCUSSION

The enzyme described in this report has specific characteristics that are not found in the other *T. denticola* peptidases or proteases. (i) The protease has specificity for phenylalanine, which indicates that it is a chymotrypsinlike enzyme. (ii) In contrast to other enzymes, the active form of the protease has a molecular weight of 95,000. Molecular weights of 29,000, 45,000, and 100,000 have been reported for *T. denticola* enzymes that hydrolyze aspartyl–*p*-nitroanilide, BAPNA, and proline–*p*-nitroanilide, respectively (14). (iii) The protease is inhibited by TPCK and also by

PMSF, a serine protease inhibitor. Its activity is increased in the presence of cysteine or dithiothreitol and is decreased by sulfhydryl reagents *p*-chloromercuribenzoic acid, iodoacetic acid, $HgCl_2$, and oxidized glutathione, which suggests that thiol groups are involved in the enzyme activity. The proline–*p*-nitroanilide-hydrolyzing enzyme has also been reported to be dependent on thiol groups. Its activity, however, was found to be unaffected by EDTA and phenanthroline and was only slightly inhibited by PMSF (14). The activity of the arginine peptidase has been reported to remain unchanged in the presence of metal chelators or sulfhydryl reagents, while PMSF, TLCK, α_1-antitrypsin, and soybean trypsin inhibitor were found to inhibit the activity (20).

The chymotrypsinlike enzyme is a true protease. It degrades a variety of proteins such as immunoglobulin G, immunoglobulin A, BSA, transferrin, α_1-antitrypsin, fibrinogen, and gelatin, and it produces either limited or multiple cleavages of the polypeptides. It is the only protease detected by the protein-conjugated SDS-polyacrylamide gel assay. Since only about 4% of the enzyme activity was recovered after the preparative gel electrophoresis procedure, it appears that much of the activity of this enzyme is lost during the procedure. Alternatively, it is possible that there is more than one chymotrypsinlike enzyme in *T. denticola*. Other enzymes which were previously described (14, 15, 20), such as the arginine and proline peptidases, apparently lose their activity under the assay conditions (SDS), as they were not detected with the BSA-gel assay. These enzymes were also reported to have weak or no activity against native proteins such as BSA. The finding that activity was removed by high-speed centrifugation and the observation that the enzyme migrates into the gel only in the presence of SDS suggest that the enzyme is associated with a particulate fraction. Possibly, the enzyme is located in the cell membrane.

A strong adherence of *T. denticola* to epithelial cells has been reported (21, 22). Both whole spirochetes and their extracts have been found to cause damage and detachment of the epithelial cells. The factor responsible appeared to be a protein requiring free sulfhydryl groups for activity. It is possible that sulfhydryl-dependent proteases such as the one we have described in this study act on the cell surface or on attachment proteins, causing the observed damage. In a preliminary study, we have observed, by using the antibody specific to the protease and by an immunofluorescence method, that the enzyme is present on the surface of the spirochetes. A factor in *T. denticola* extracts inhibiting blastogenesis of lymphocytes has been described elsewhere (24). It was suggested that the factor is a protein with a molecular weight of about 100,000. Again, it is possible that the spirochete proteases exert immunosuppressive effects. These possibilities are currently under investigation in our laboratory. *T. denticola* has also been shown to possess enzymes that degrade glycosaminoglycans such as hyaluronic acid and chondroitin sulfate (4). With these enzymes, the proteases may be important in the invasiveness of the spirochetes. Indeed, spirochetes have been found to be predominant among the bacterial groups that invade gingival connective tissue in acute necrotizing ulcerative gingivitis (10).

There are a number of other ways by which the protease we have described here can cause tissue damage and participate in the pathogenic process of periodontal diseases. The ability of the protease to degrade immunoglobulin A, immunoglobulin G, α_1-antitrypsin, and other serum proteins may be a virulence factor by which the spirochete escapes the host defense mechanisms and disrupts the metabolism of periodontal tissue.

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LITERATURE CITED

1. **Armitage, G. C., W. R. Dickinson, R. S. Jenderseck, S. M. Levine, and D. W. Chambers.** 1982. Relationship between the percentage of subgingival spirochetes and the severity of periodontal disease. J. Periodontol. **53**:550–556.
2. **Boehringer, H., N. S. Taichman, and B. J. Shenker.** 1984. Suppression of fibroblast proliferation by oral spirochetes. Infect. Immun. **45**:155–159.
3. **Cheng, S.-L., and E. C. S. Chan.** 1983. The routine isolation, growth and maintenance of the intermediate-size anaerobic oral spirochetes from periodontal pockets. J. Periodontal Res. **18**: 362–368.
4. **Fiehn, N.-E.** 1986. Enzyme activities from eight small-sized oral spirochetes. Scand. J. Dent. Res. **94**:132–140.
5. **Foltmann, B., P. B. Szecsi, and N. I. Tarasova.** 1985. Detection of proteases by clotting of casein after gel electrophoresis. Anal. Biochem. **146**:353–360.
6. **Frank, R. M.** 1980. Bacterial penetration in the apical pocket wall of advanced human periodontitis. J. Periodontal Res. **15**: 563–573.
7. **Kelleher, P. J., and R. L. Juliano.** 1984. Detection of proteases in polyacrylamide gels containing covalently bound substrates. Anal. Biochem. **136**:470–475.
8. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
9. **Laughon, B. E., S. A. Syed, and W. J. Loesche.** 1982. API ZYM system for identification of *Bacteroides* spp., *Capnocytophaga* spp., and spirochetes of oral origin. J. Clin. Microbiol. **15**:97–102.
10. **Listgarten, M. A.** 1965. Electron microscopic observations of the bacterial flora of acute necrotizing ulcerative gingivitis. J. Periodontol. **36**:328–339.
11. **Listgarten, M. A.** 1976. Structure of the microbial flora associated with periodontal health and disease in man. A light and electron microscopic study. J. Periodontol. **47**:1–18.
12. **Listgarten, M. A., and L. Hellden.** 1978. Relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans. J. Clin. Periodontol. **5**:115–132.
13. **Loesche, W. J., S. A. Syed, E. Schmidt, and E. C. Morrison.** 1985. Bacterial profiles of subgingival plaques in periodontitis. J. Periodontol. **56**:447–456.
14. **Makinen, K. K., S. A. Syed, P.-L. Makinen, and W. J. Loesche.** 1986. Benzoylarginine peptidase and iminopeptidase profiles of *Treponema denticola* strains isolated from the human periodontal pocket. Curr. Microbiol. **14**:85–89.
15. **Makinen, K. K., S. A. Syed, P.-L. Makinen, and W. J. Loesche.** 1987. Dominance of iminopeptidase activity in the human oral bacterium *Treponema denticola* ATCC 35405. Curr. Microbiol. **14**:341–346.
16. **Mikx, F. H. M., and M. H. De Jong.** 1987. Keratinolytic activity of cutaneous and oral bacteria. Infect. Immun. **55**:621–625.
17. **Mikx, F. H. M., D. N. B. Ngassapa, F. M. J. Reijntjens, and J. C. Maltha.** 1984. Effect of splint placement of black-pigmented *Bacteroides* and spirochetes in the dental plaque of Beagle dogs. J. Dent. Res. **63**:1284–1288.
18. **Moore, W. E. C., L. V. Holdeman, E. P. Cato, R. M. Smibert, J. A. Burmeister, K. G. Palcanis, and R. R. Ranney.** 1985. Comparative bacteriology of juvenile periodontitis. Infect. Immun. **48**:507–519.

19. **Nitzan, D., J. F. Sperry, and T. D. Wilkins.** 1978. Fibrinolytic activity of oral anaerobic bacteria. Arch. Oral Biol. **23**:465–470.
20. **Ohta, K., K. K. Makinen, and W. J. Loesche.** 1986. Purification and characterization of an enzyme produced by *Treponema denticola* capable of hydrolyzing synthetic trypsin substrates. Infect. Immun. **53**:213–220.
21. **Olsen, I.** 1984. Attachment of *Treponema denticola* to cultured human epithelial cells. Scand. J. Dent. Res. **92**:55–63.
22. **Reijntjens, F. M. J., F. H. M. Mikx, J. M. L. Wolters-Lutgerhorst, and J. C. Maltha.** 1986. Adherence of oral treponemes and their effect on morphological damage and detachment of epithelial cells in vitro. Infect. Immun. **51**:642–647.
23. **Saglie, R., M. G. Newman, F. A. Carranza, and G. L. Pattison.** 1982. Bacterial invasion of gingiva in advanced periodontitis in humans. J. Periodontol. **53**:217–222.
24. **Shenker, B. J., M. A. Listgarten, and N. S. Taichman.** 1984. Suppression of human lymphocyte responses by oral spirochetes: a monocyte-dependent phenomenon. J. Immunol. **132**: 2039–2045.
25. **Uitto, V.-J.** 1983. Degradation of basement membrane collagen by proteinases from human gingiva, leukocytes and bacterial plaque. J. Periodontol. **54**:740–745.
26. **Uitto, V.-J., E. C. S. Chan, and T. Chin Quee.** 1986. Initial characterization of neutral proteinases from oral spirochetes. J. Periodontal Res. **21**:95–100.