New Pepsin-Solubilized Low Molecular Weight Collagenous Component Possibly Unique to Periodontal Ligament†

Mitsuo Yamauchi,* Yoshinori Kuboki,‡ Satoshi Sasaki,§ and Gerald L. Mechanic

Dental Research Center and Department of Biochemistry and Nutrition, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

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ABSTRACT: Limited pepsin digestion of bovine periodontal ligament releases genetic types I, III, and V collagen and a high cystine containing low molecular weight collagenous component. Salt fractionation and molecular sieve chromatography allowed the isolation of the latter as an apparently pure homogeneous moiety which had an approximate molecular mass of 30 000 daltons. Reduction with mercaptoethanol yielded a single 10 000-dalton band on polyacrylamide gel electrophoresis in sodium dodecyl sulfate. This led us to conclude that the newly isolated low molecular weight collagen fragment consists of three similar molecular weight chains. Unreduced collagen-like glycoprotein (CGP) [Jander, R., Troyer, D., & Rauterberg, J. (1984) *Biochemistry 23*, 3675–3681] after extraction from tissues with collagen denaturing solvents yields the GP140 glycoprotein upon reduction and does not release any collagen fragment below 90 000 daltons upon mild or vigorous pepsin digestion. The GP140 glycoprotein [Heller-Harrison, R. A., & Carter, W. G. (1984) *J. Biol. Chem. 259*, 6858–6864] isolated by extraction under reducing and collagen denaturing solvent conditions did not yield a collagen fragment below 40 000 daltons after pepsin treatment. It was clearly shown that both CGP and GP140 yield type VI collagen fragments in the above-cited reports. Since this report demonstrates that the M_r 30 000 collagen fragment is only released by pepsin treatment of nondenaturing solvent treated periodontal ligament and that only very small peptides are found in denaturing solvent treated tissue after pepsin digestion, it is concluded that the newly isolated M_r 30 000 collagen fragment reported here is not derived from type VI collagen. In support of this conclusion, peptide mapping of complete tryptic digests by reversed-phase chromatography indicates significant differences between the M_r 30 000 collagen fragment and types VI and IX collagen. Further support for the new collagen fragment was obtained by amino acid compositional data as well as analyses for carbohydrated hydroxylysine. Molecular composition and other characteristics suggest it is a portion of an insoluble highly cross-linked larger molecular weight collagen consisting of discontinuous triple-helical, globular, and high disulfide cross-linked globular domains. It is also suggested that this collagenous component is unique to periodontal ligament and represents another as yet undescribed genetic type of collagen.

The periodontal ligament is the soft connective tissue situated between the cementum of the tooth and the alveolar bone forming the socket wall. The principal functions of this tissue are to support a tooth in its socket and to withstand the considerable forces of mastication. These important functions largely depend on the physicochemical properties of the macromolecular components of the connective tissue matrix which mainly consist of collagenous fibers (Saito, 1960). Previous studies on the collagen of this tissue showed its exceptionally high rate of turnover (Sodek, 1977) and the similarity of the pattern of reducible cross-links to that of skin collagen (Kuboki et al., 1981).

All of the presently known genetic types of collagen except for type I have been isolated as soluble proteins by the now classical method of limited pepsin digestion of a variety of collagen-containing tissues. Their successful solubilization and subsequent salt fractionation have allowed investigators to purify as well as characterize each one of this important group of proteins. Many of these collagens except for types I, II, and IV have as yet still unknown physiological functions.

Limited pepsin digestion of the collagen of periodontal ligament indicated the presence of types I and III collagen (Butler et al., 1975). The latter report also demonstrated that 20% was type III and the remainder type I by analysis of the ligament's cyanogen bromide derived peptides. No other collagen type was or has been reported from this tissue.

Recently, within the past 3 or 4 years, a number of cystine-containing low molecular weight collagenous components have been isolated from several tissues. Direct extraction and salt fractionation from pepsin-treated cartilage have yielded proteins of apparent molecular weights of 30 000–59 000 (Reese & Mayne, 1981; Von Der Mark et al., 1982; Shimokomaki et al., 1981; Ayad et al., 1982; Ricard-Blum et al., 1982). No collagen in this molecular weight range has been isolated from placenta without reduction of the cystine residues (Jander et al., 1981; Odermatt et al., 1983). Careful investigation of fetal and mature nuchal ligament has also failed to reveal a low molecular weight collagen in the range stated above (Chambers et al., 1984; Knight et al., 1984).

The present paper describes the isolation of a heretofore undescribed collagen component from bovine periodontal ligament. The new low molecular weight collagen (M_r 30 000) will be referred to as LMCP,[1] and careful review of the lit-

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* Correspondence should be addressed to this author at the Dental Research Center, The University of North Carolina at Chapel Hill.

‡ Present address: Dental Clinical Laboratory, Tokyo Medical and Dental University.

§ Present address: Department of Biochemistry, School of Dentistry, Tokyo Medical and Dental University.

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erature suggests that this collagen might be unique to periodontal ligament.

Experimental Procedures

Preparation of Collagen of Periodontal Ligament. Periodontal ligamental tissues were obtained as previously described (Butler et al., 1975). All procedures were carried out at 4 °C. The fresh tissue (15 g wet weight, 3.1 g lyophilized dry weight) was suspended and homogenized, by using a Polytron homogenizer, in approximately 10 volumes of distilled water containing 0.01 M disodium ethylenediaminetetraacetate, 0.005 M *N*-ethylmaleimide, 0.0005 M phenylmethanesulfonyl fluoride, and 0.025 M ϵ-aminocaproic acid as protease inhibitors. The homogenate was centrifuged at 10000*g*, and the precipitate was washed repeatedly (5 times) with the same solution. The residue was suspended in 200 mL of 0.5 M acetic acid and treated with pepsin (0.5 mg/mL, Worthington) for 24 h at 4 °C. The pepsin-solubilized material was separated from the insoluble residue by centrifugation at 10000*g* for 30 min, and the supernatant was made 0.7 M in NaCl. The resulting precipitate was removed by centrifugation at 20000*g* for 1 h. Salt was added to the resulting supernatant until it was 1.2 M and centrifuged again. The supernatant was then made 2.0 M with respect to NaCl. The precipitate was collected as just described. This was then exhaustively dialyzed against 0.1 M acetic acid and lyophilized. The lyophilized 2 M NaCl precipitate was dissolved in 5 M guanidine hydrochloride and 0.05 M Tris-HCl, pH 7.5, and denatured by warming for 30 min at 55–60 °C. This solution was applied to a column (2.5 × 200 cm) of Sepharose 4B equilibrated and eluted with 2 M guanidine hydrochloride and 0.05 M Tris-HCl, pH 7.5, at a flow rate of 20 mL/h. The main fraction was recovered from Sepharose 4B column chromatography, dialyzed against distilled water, and lyophilized. This material was dissolved as described above and applied to a column (1.2 × 110 cm) of Bio-Gel A-0.5m equilibrated with the same buffer as described above and chromatographed at a flow rate of 8 mL/h. The main component recovered from this was treated as described above, applied to a column (1.5 × 90 cm) of Sephadex G-75 superfine, and chromatographed at a flow rate of 6 mL/h using the same buffer system as above.

CM-cellulose Chromatography. Chromatography of the LMCP was attempted on CM-cellulose. The lyophilized protein from above was dissolved in 0.04 M sodium acetate buffer at pH 4.8 which contained 2 M urea and 1% 2-propanol (Fukae & Mechanic, 1980). The column (1.5 × 12 cm) was equilibrated with the same buffer solution at 45 °C.

Amino Acid Analysis. Samples were hydrolyzed in constant-boiling HCl and 2 N NaOH at 110 °C for 24 h in an atmosphere of N_2. Hydrolysates were then analyzed with a Hitachi 835 amino acid analyzer and a Varian 5560 HPLC configured as an amino acid analyzer using ninhydrin at 135 °C as a postcolumn detection system.

Polyacrylamide Gel Electrophoresis (PAGE). Characterization of the protein was carried out by sodium dodecyl sulfate (SDS)–PAGE using a Pharmacia gradient PAA4/30 gel with and without reduction by 5% 2-mercaptoethanol. Electrophoresis buffer was 0.04 M Tris, 0.02 M sodium acetate, and 0.002 M EDTA, pH 7.4, containing 0.2% SDS. Protein bands were stained with 0.5% Coomassie Brilliant Blue R-250 in 30% methanol and 10% acetic acid. After gels were destained in 10% methanol and 10% acetic acid, protein bands were scanned at 570 nm by using a FD-AIV (FUJIRIKEN) scanning densitometer.

CNBr Treatment. The LMCP was reacted with CNBr in the presence of 70% formic acid as previously described (Epstein et al., 1971). The collagen was also treated in the same manner at 37 °C after reduction with 25% mercaptoethanol as described earlier (Adelstein & Kuehl, 1970).

Collagenase Digestion. Dried aliquots of protein were treated with apparently pure bacterial collagenase (Advanced Biofacture, form III) (enzyme:substrate ratio = 1:100) in 0.0125 M Tris, pH 7.5, containing 0.03 M *N*-ethylmaleimide and 0.006 M $CaCl_2$. Digestion was carried out for 15 h at 37 °C. Collagenase action was stopped by addition of 0.5 M EDTA and 0.05 M Tris-HCl, pH 7.5, and heated for 10 min at 100 °C.

Guanidine Extraction of Periodontal Ligament. Five grams of lyophilized untreated periodontal ligament was extracted with 500 mL of 6 M guanidine hydrochloride and 0.05 M Tris-HCl, pH 7.4, for 3 days at 4 °C with stirring with and without 1,4-dithiothreitol.

The suspension was centrifuged, and the insoluble material was reextracted at room temperature with 200 mL of the same solution. The supernatant from the cold extraction was dialyzed exhaustively against water and lyophilized. The same procedures were used to obtain the denatured collagen from the extraction at room temperature.

The guanidine hydrochloride extracts and their residues were subjected to limited pepsin digestion as described above for the native tissue.

Peptide Mapping. Complete tryptic digests were prepared from reduced LMCP, genetic type IX, and the three pepsin-resistant components of type VI collagen after denaturation in 0.2 M NH_4HCO_3 (pH 7.9) by heating to 65 °C for 15 min as reported previously (Becker et al., 1975). The trypsin treatment completely solubilized all the proteins which were insoluble even after the heat denaturation step. Type VI and IX collagens were generously supplied by Dr. D. Furuto. Each digest was subjected to HPLC reversed-phase chromatography using a Vydac C_{18} 300-Å pore column. The gradient was made between 0.025 M phosphate buffer, pH 2.85, and 50% acetonitrile in a linear fashion by using a Varian 5560 liquid chromatograph at 1 mL/min. The UV monitor on the instrument was set at 230 nm. Approximately 0.110 mg of LMCP peptides and 0.330 mg of the peptides derived from the other collagen were applied to the column.

Results

Limited pepsin digestion solubilized approximately 90% of the periodontal ligament. Salt fractionation yielded 1.3 g of material in the 0.7 M NaCl precipitate; 5.2 mg was present in the 1.2 M NaCl precipitate and 8.5 mg in the 2.0 M NaCl precipitate. PAGE in SDS under reducing and nonreducing conditions indicated the 0.7 M NaCl precipitate contained types I and III collagen while the 1.2 M NaCl precipitate contained genetic types I and V collagens. Examination of the 2.0 M NaCl precipitate obtained from the original 0.5 M acetic acid collagen solution by PAGE in SDS indicated its most abundant proteinaceous material had an R_f that corresponded to a molecular weight of 30 000.

Molecular Sieve Chromatography. The protein that was recovered from the 0.5 M acetic acid 2.0 M NaCl precipitate as described above was chromatographed on a calibrated column of Sepharose 4B in 2.0 M guanidine hydrochloride and 0.05 M Tris-HCl, pH 7.4. A representative elution profile is depicted in Figure 1A and shows the presence of nine peaks

[1] Abbreviations: LMCP, M_r 30 000 collagen from periodontal ligament; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; CNBr, cyanogen bromide; CGP, collagen glycoprotein; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; HPLC, high-performance liquid chromatography; EDTA, ethylenediaminetetraacetic acid.

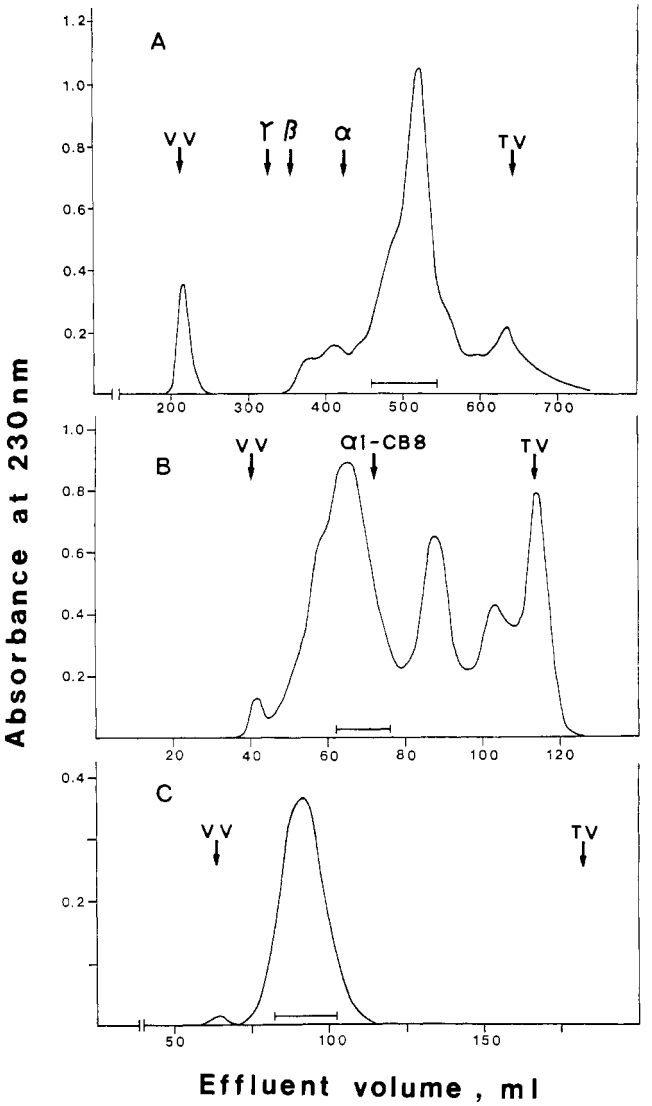

FIGURE 1: Chromatography of the 2.0 M NaCl precipitate from the 0.5 M acetic acid pepsin-solubilized collagens. These are successive molecular sieve elution profiles for (A) Sepharose CL-4B, (B) Bio-Gel A-0.5m, and (C) Sephadex G-75 superfine. Conditions for chromatography are in the text. Arrows denote elution positions of known collagen standards with VV denoted as the void volume, γ components (M_r 285 000), β components (M_r 190 000), α chains (M_r 95 000), TV as the total column volume, and α1-CB8 (M_r 25 100). Fractions pooled are represented by bars.

and shoulders with the first being eluted at the void volume. The fractions denoted by the bar were pooled and rechromatographed in the same solvent on a calibrated column of Bio-Gel A-0.5m after it was discerned that the protein of interest (30 000 daltons) was in this peak as determined by PAGE in SDS. A typical profile from the A-0.5m column of this fraction is shown in Figure 1B. PAGE in SDS indicated that the 30 000-dalton material was in the fractions signified by the bar in Figure 1B. The pooled fractions were finally chromatographed, by using the same solvent, on a column of Sephadex G-75 superfine, and the resulting pattern is shown in Figure 1C.

CM-cellulose Chromatography. The fractions over the bar from Figure 1C were desalted, lyophilized, and dissolved in buffer as described under Experimental Procedures and applied to the column. This procedure was unsuccessful for the protein was unretained by the column. This indicated the LMCP was acidic in nature.

Polyacrylamide Gel Electrophoresis. The PAGE pattern in SDS for the major peak from the Sephadex G-75 column (Figure 1C) indicated a single band. The gel exhibited an apparently homogeneous protein. This band had an apparent mobility slightly less than that of α1CB8. A plot of its relative mobility with the known CNBr peptides against molecular weight indicated an apparent molecular weight of 30 000 for the single band. This was consistent with the molecular weight obtained from the gel filtration columns. Reduction with 2-mercaptoethanol and subsequent electrophoresis under the same conditions yielded a single band with a mobility apparently equal to approximately 10 000 daltons. In some preparations, a small amount of material (about 10%) appeared at a relative mobility that corresponded to 20 000 daltons, indicating incomplete reduction or some reassociation. This has also been observed with other high cystine containing collagenous components (Furuto & Miller, 1980).

None of the guanidine hydrochloride extracts of native periodontal ligament contained a band with an R_f of 30 000 daltons on PAGE in SDS. In addition, no bands were obtained that corresponded to the CGP or the GP140 glycoproteins that have been shown to yield type VI collagenous components after pepsin treatment (Heller-Harrison & Center, 1984; Jander et al., 1984). PAGE of the limited pepsin digests of the guanidine hydrochloride extracts and their residue indicated complete degradation to peptides of 3000 daltons and below for the extracts and the residue. This indicated that guanidine hydrochloride denatured all the collagen in periodontal ligament.

Amino Acid Composition of LMCP. Amino acid analyses of acid hydrolysates revealed an unusual composition and is shown in Table I. Identical results were obtained with three other preparations of LMCP. Glycine is markedly low while hydroxyproline as well as hydroxylysine is in accord with other collagens. High levels of serine, threonine, proline, and aspartic acid are present. Tyrosine is also a bit high when viewed against other collagens, and isoleucine is higher than leucine which is another unusual feature. The most unusual feature is the very high content of cystine. Alkaline hydrolysis indicated that 44% of the Hyl was diglycosylated, 34% was monoglycosylated, and 22% was unglycosylated. This differs from the type VI collagen in which Hyl is entirely glycosylated (Furuto & Miller, 1980).

CNBr Digestion. CNBr digestion indicated no change in mobility on PAGE of the LMCP although methionines are present in the molecule (Table I). Reduction with 2-mercaptoethanol and subsequent digestion with CNBr demonstrated as well no change in the mobility of the M_r 10 000 band on PAGE in SDS.

Collagenase Treatment of LMCP. Digestion of the LMCP (Figure 2, lane 5) with bacterial collagenase was complete as adjudged by PAGE in SDS (Figure 2, lane 6). Figure 2 also depicts the results obtained by collagenase digestion of type I collagen and α1(I) chain from bovine periodontal ligament. Note type I collagen (Figure 2, lane 1) was completely digested with collagenase (Figure 2, lane 2) as was the α1(I) chain (Figure 2, lane 4) which is exhibited in Figure 2, lane 3, before digestion. It was estimated that digestion produced fragments below M_r 5000.

Peptide Mapping. The reversed-phase HPLC chromatographic patterns of the tryptic peptides derived from LMCP, and the three collagenous components from genetic type VI collagen, are depicted in Figure 3. The pattern derived from type IX collagen is not shown because it was markedly different when compared to the patterns obtained in Figure 3. It must be noted that significant differences are evident between the peptide maps. The very large peak with a retention

Table I: Amino Acid Compositions of LMCP and Other Pepsin-Derived Collagen Fragments

		type VI[c]		type IX[d]	
	LMCP[e] residues/1000	acidic[e] residues/1000	basic[f] residues/1000	high mol wt residues/1000	low mol wt residues/1000
Hyp	89	86	69	95	105
Asp	66	83	72	53	50
Thr[a]	34	12	16	16	20
Ser[a]	65	23	28	38	22
Glu	91	114	98	97	81
Pro	134	100	107	90	92
Gly	225	293	304	323	310
Ile	31	20	19	27	24
Leu	28	23	23	48	53
Tyr	10	23	22	5	6
Phe	15	17	14	8	8
Hyl	9	28	39	34	20
Lys	12	3	2	21	20
His	12	11	18	8	10
Arg	43	53	69	46	66
$^1/_2$-Cys[b]	69	31	36	2	9
total	1000	1000	1000	1000	1000
Glc-Gal-Hyl	4	23	34		
Gal-Hyl	3	5	5		
Hyl	2	0	0		

[a] Uncorrected for destruction on hydrolysis. [b] Determined as cysteic acid (Hirs, 1967). [c] Furuto & Miller (1980). [d] Reese & Mayne (1981). [e] Unretained by CM-cellulose. [f] Retained by CM-cellulose.

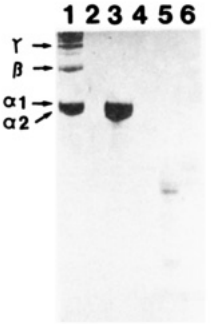

FIGURE 2: SDS–polyacrylamide gel electrophoresis of collagens with and without treatment with bacterial collagenase. (1) Type I collagen from bovine periodontal ligament; (2) same sample after enzyme treatment; (3) α1 chain of type I collagen from bovine periodontal ligament; (4) same sample after enzyme treatment; (5) LMCP; (6) same sample after enzyme treatment.

time of 9.425 min present in LMCP (Figure 3A) is absent from the CM-cellulose acidic (unretained) component of type VI collagen (Figure 3B) (Furuto & Miller, 1981). In addition, the group of peaks from retention times 72.494–76.145 min are also absent from the latter pattern. It must be remembered that LMCP is unretained by CM-cellulose. The same type of analysis may be made for the patterns depicted in Figure 3C,D. The latter two are type VI derived collagens that are retained by CM-cellulose (Furuto & Miller, 1980).

Discussion

The study documented in this report is a simple procedure for the reproducible isolation and purification of a collagenous protein which is obtained by limited pepsin digestion of whole bovine periodontal ligament. This protein is a relatively low molecular weight (M_r 30000) aggregate which upon reduction yields a single 10000-dalton band on PAGE in SDS. Presumably the aggregate contains three M_r 10000 chains that are stabilized by interchain disulfide bonds. This peridontal ligament derived collagen comprises approximately 1% of the total amount of the collagen components solubilized from the tissue as determined by fractionation and final weight.

We have used relatively mild conditions to extract LMCP from periodontal ligament; the conditions were similar to those used to obtain type IX collagen from cartilage, that is, pepsin treatment for 24 h at 4 °C. Considerably harsher conditions have been used to obtain type VI collagen fragments from placenta. Odermatt et al. (1981) used pepsin for 24 h at room temperature in one of their procedures and obtained collagen fragments considerably larger than LMCP. Others have also used conditions of pepsin treatment from 20 to 60 h at 4 °C to obtain type VI fragments (Jander et al., 1981; Traueb & Bornstein, 1984). Needless to say, similar type VI collagen fragments are obtained by all the various procedures cited. In all cases, only upon reduction with 2-mercaptoethanol is a fragment or chain of M_r 32000–40000 obtained with type VI collagen (Jander et al., 1981; Odermatt et al., 1983; Furuto & Miller, 1980, 1981; Traueb & Bornstein, 1984). The collagenous fragment reported here yields completely different results.

Ayad et al. (1982) treated cartilage with pepsin for 48 h and isolated a short-chain disulfide-bonded collagen after salt fractionation and one pass through an agarose A-5m column. Its reported weight was M_r 36000 and upon reduction yielded three separable chains of M_r 16000, 10000, and 8000, respectively, on PAGE in SDS. The unreduced molecule possessed an entirely different amino acid composition from LMCP (Ayad et al., 1982). Another group of investigators (Von Der Mark et al., 1982) obtained a similar short-chain collagen from the same tissue and reported a weight of M_r 32000 which on reduction now only yielded two fragments, one of M_r 12000 and the other M_r 10000, on PAGE in SDS. Its amino acid composition was similar to that reported by Ayad et al. (1982). Reese and Mayne (1981), using similar procedures, isolated from cartilage a low molecular weight disulfide-bonded collagen of M_r 30000 which on reduction yielded two chains, M_r 20000 and 10000. The unreduced collagen, although similar in weight to LMCP but different in chemical characteristics, possessed an entirely different amino acid composition (see Table I).

Digestion of unreduced LMCP with bacterial collagenase fragmented the collagen to low molecular weight. This in-

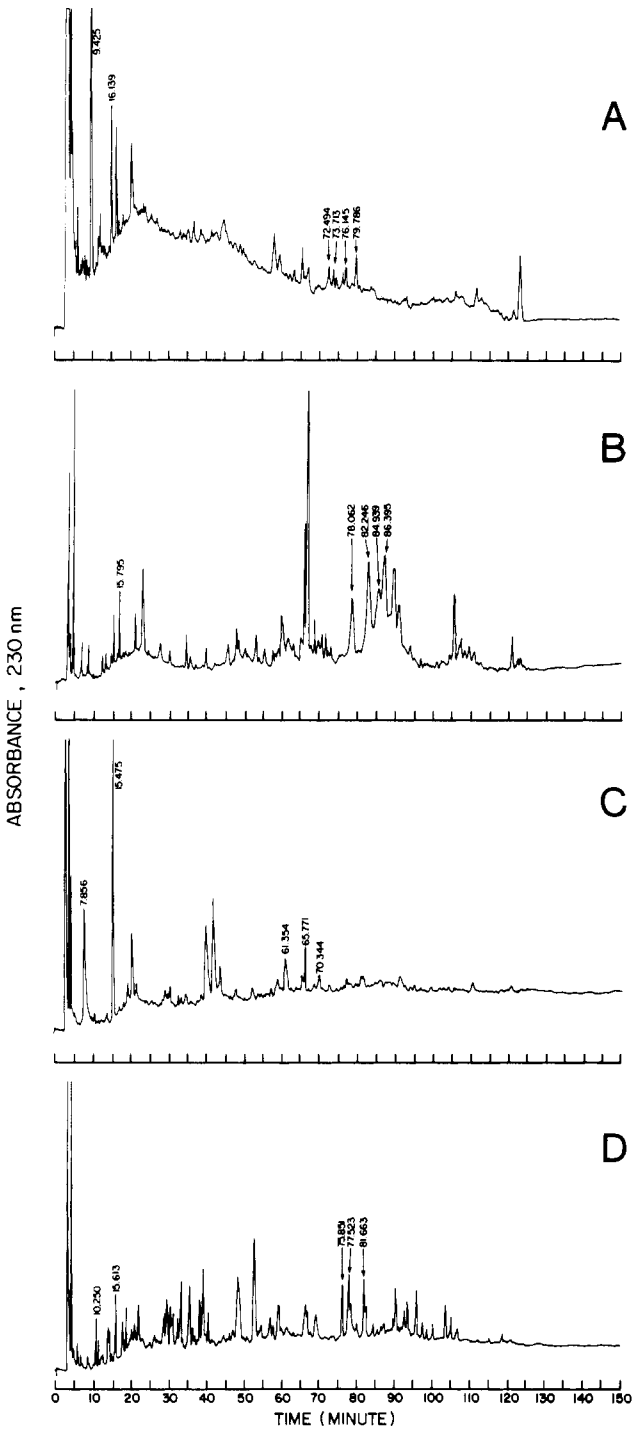

FIGURE 3: Comparison of tryptic peptide maps by reversed-phase HPLC from (A) LMCP and (B) the acidic component from type VI collagen which is unretained by CM-cellulose. (C and D) Basic components from type VI collagen which are retained by CM-cellulose. (C) represents the leading shoulder, and (D) represents the main peak of the more basically charged collagen fragments of type VI collagen. [see Furuto & Miller (1980)].

dicates a significant and unequivocal difference between LMCP and type VI collagen (see Figure 2). Treatment of type VI collagen with the same enzyme for a longer period of time under the same conditions essentially had no effect (Odermatt et al., 1983), Furuto & Miller, (1981). When type VI collagen was heat denatured then, some effect was noted in that the major fragment had a mobility between β and γ on PAGE in SDS (Odermatt et al., 1983). Only when type VI collagen was reduced and then treated with collagenase could an effect be seen in that the products migrated toward the bottom of the gel on 5% PAGE in SDS (Furuto & Miller, 1981).

LMCP contains methionine residues (Table I), yet reduction with 2-mercaptoethanol followed by treatment with CNBr did not alter the mobility of the M_r 10000 band on PAGE in SDS (data not shown). This indicates that the methionines are located close to either end of the molecule since no discernible change in molecular weight was noted.

The rationale for using guanidine hydrochloride extraction of whole periodontal ligament, with and without 1,4-dithiothreitol, was to explore the possibility that LMCP occurred naturally as an in vivo degradation product because of the tissue's documented high turnover rate (Sodek, 1977). We failed to find LMCP by this method and therefore have come to the conclusion that LMCP is a true product of limited pepsin digestion and is derived from a larger molecular weight insoluble collagen component in periodontal ligament.

Pepsin treatment of denaturing solvent treated periodontal ligament resulted in degradation to small peptides. However, this behavior is not the case with type VI collagen. For instance, the collagen-like glycoprotein (CGP), the precursor of type VI collagen fragments, was extracted from calf aorta and ligamentum nuchae by using 6 M urea at room temperature (Jander et al., 1984), a treatment that surely denatures the usual collagen. The pepsin-derived products of the CGP upon being reduced yielded the type VI collagen fragments SC1, SC2, and SC3 reported previously (Jander et al., 1981). These presumably single chains of short-chain collagen are larger than the three-chain LMCP. This behavior is clearly quite distinct from the chemical characteristics exhibited by LMCP from periodontal ligament. The same authors (Jander et al., 1984) showed that glycoprotein GP140 is derived from CGP. In demonstrating that pepsin-generated type VI collagen is a degradation product of GP140, an extracellular matrix glycoprotein, Heller-Harrison and Carter (1984) extracted the GP140 from placentas with 8 M urea and 0.025 M dithiothreitol. Following digestion with pepsin, the SC1, SC2, and SC3 chains of type VI collagen were isolated. Here again is a case under whose conditions LMCP from periodontal ligament does not survive as peptides greater than M_r 3000. Finally, Traueb and Bornstein (1984) pepsin treated a 6 M guanidine hydrochloride extract of bovine uterus as well as aorta in order to isolate the SC1, SC2, and SC3 chains of type VI collagen. Furthermore, none of the immunoblot nor Coomassie Blue stained gels of the pepsin-treated unreduced CGP indicated any band below M_r 40000 (Jander et al., 1984). The same was true with the GP140 collagen-like glycoprotein (Heller-Harrison & Carter, 1984). In other words, none of the pepsin treatments, whether harsh or mild, in any of the reports on type VI collagen yielded fragments below M_r = 40000 (Heller-Harrison & Carter, 1984; Jander et al., 1984; Traueb & Bornstein, 1984). It is very clear from the discussion above that LMCP is not in any way derivable from type VI collagen. To obtain LMCP from periodontal ligament, the latter must not be subjected to the usual collagen denaturing conditions before pepsin treatment; otherwise, essentially degraded collagen is obtained in the form of peptides below M_r 3000 as reported here.

The LMCP aggregate is an acidic protein. This is the only resemblance it has to one of the type VI collagen fragments isolated by Furuto and Miller (1981).

The compositional features of LMCP differ significantly in several respects from those of any collagen reported previously (Table I). Alkaline hydrolysis of LMCP followed by amino acid analysis indicated mono-, di-, and unglycosylated

Hyl in the protein (Table I). This is another distinguishing feature of LMCP when compared to type VI collagen which contains all of its hydroxylysine in the glycosylated form (Furuto & Miller, 1980). The most remarkable feature is the unusually high content of half-cystine which to date is the largest amount reported in any isolated collagen fragment. Even so, this substantial amount of cystine did not protect LMCP from degradation by bacterial collagenase which is the case for type VI collagen (Furuto & Miller, 1980).

The composition of LMCP and its ready degradation by bacterial collagenase clearly indicate it is a collagen-like protein. Its other chemical characteristics distinguish it from types VI and IX and indicate that this is a new and unique collagen.

In order to support our contention that LMCP is a new and unique collagen fragment from periodontal ligament and that it is not derived from but is different from both type VI and type IX collagens, we have compared the peptide maps of the complete tryptic digests of reduced LMCP, reduced type VI (Furuto & Miller, 1981), and type IX collagen fragments by reversed-phase chromatography (see Figure 3 and Results). It is quite evident from the data that LMCP is different from and is not derived from type VI collagen. The pattern for type IX was so different it is not presented in the interest of saving space. The interpretation of the tryptic peptide map data is unequivocal and clearly demonstrates that many of the peptides derived from LMCP contain different sequences of amino acids than those present in type VI collagen. Peptide mapping is a much powerful method of analysis than could have been obtained by further attempted separation and characterization of the individual chains of LMCP.

The molecular organization of this collagen component from the data presented has some interesting aspects. The unusually low amount of glycine, which is less than one-fourth of its total amino acid composition, indicates this collagen must contain a considerable domain for its molecular weight class which does not participate in a typical collagen triple-helix structure. Perhaps this domain is next to a typical triple-helical domain and owes its pepsin resistance to the extensive intra- and interchain cystine bondings. It is most likely that a pepsin-sensitive globular domain abuts the triple-helical domain on its other side and is part of a higher molecular weight collagenous component. This is suggested because of the guanidine hydrochloride extraction data and the difficulty of envisioning so short a triple-helical domain as LMCP possesses as being part of a collagenous structural protein that has such a stressful and important support function. We would like to suggest that LMCP is derived from a much higher molecular weight collagen that is characterized by discontinuous triple-helical, globular, and high disulfide cross-linked domains. As reported here, we have attempted to isolate the parent collagen of LMCP; however, we did not succeed by trying any of the known methods of isolating a parent collagen from its original tissue. In spite of the latter, it is quite clear from the experimental data that LMCP represents another as yet unknown collagen.

The compositional features of the LMCP also eliminate its arising from any of the procollagen extension peptides of type I or III collagen (Olsen et al., 1977; Bruckner et al., 1978) from $C1_q$ complement (Reid, 1979) or acetylcholinesterase (Rosenberry et al., 1982).

The characterization of this new low molecular weight collagen suggests that this new collagenous component might possibly be unique to periodontal ligament although no functional role can at the present time be attributed.

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