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Characterization of Collagens of Diseased Human Gingiva†

A. Sampath Narayanan,* Roy C. Page, and David F. Meyers

ABSTRACT: In the gingiva and other connective tissues, alteration in the collagens is primarily responsible for their functional impairment during disease. To study the collagen alterations, we extracted diseased human gingival tissue with neutral and acidic solvents and then with pepsin. The pepsin extract was separated into proteins soluble in 2.5 and 1.5 M NaCl and proteins insoluble in 1.5 M NaCl. By the criteria of solubility behavior in NaCl solutions, elution from (carboxymethyl)cellulose (CM-cellulose) columns, sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gel electrophoresis, CNBr peptide pattern, and amino acid composition, the collagens of acidic and neutral solvent extracts and 1.5 M soluble fraction of pepsin extract were characterized as type I collagen and the 1.5 M NaCl insoluble collagen as type III. The 2.5 M NaCl fraction contained α1, A, and B chains. The α1 chains resembled α1[I] in amino acid composition, and, since α2 chains were lacking, it appeared that these chains derived from type I trimer collagen. The A and B chains were purified from the 2.5 M NaCl fractions by salting out at acidic pH. The final (A plus B) chain fraction was resolved into two major and one minor protein peaks by phosphocellulose chromatography. The major peaks were characterized as A and B chains on the basis of amino acid composition and CNBr peptide patterns. The minor peak had electrophoretic mobility slightly less than B chains, and the amino acid composition was different. Analysis of the proportion of different collagen types extracted indicated that type III collagen, which is the second major fraction in other connective tissues, is only a minor constituent in the gingiva. More interestingly, A and B chains accounted for a greater proportion than type III. Unlike the fibroblast cultures, the type I trimer formed only a small proportion of collagens of diseased gingival tissue.

The connective tissue component of normal human gingiva is made up predominantly of collagen and proteoglycans, and these components are responsible for the tensile strength, tooth supporting property, and stability of the gingiva. The gingival collagen has an unusually high degree of structure, and it is organized into distinct structural and functional fiber groups (Goldman, 1951; Arnim & Hagerman, 1953; Page et al., 1974). Analysis of CNBr digests indicated that the gingival connective tissue contains predominantly type I collagen together with small amounts of type III (Ballard & Butler, 1974; Dabbous & Brinkley, 1977), but, in contrast to skin, the gingival collagen turns over at an inordinately high rate, even in adulthood (Page & Ammons, 1974). So far systematic characterization studies to identify additional collagen chains, such as A and B,[1] in the gingiva have not been attempted, and whether the various fibers are made up of the same or different collagens remains unknown.

The collagenous component of the gingiva undergoes severe quantitative and qualitative alterations during the development of gingivitis and periodontitis, and these alterations result in functional impairment of the gingival tissues [for reviews, see Page & Schroeder (1973, 1976)]. At an early stage, ~70% of the collagen immediately adjacent to the junctional epithelium is lost, and the resident fibroblasts manifest features of cytopathic alteration. As the disease progresses, fibrosis and scarring may occur. Fairly extensive studies have been done with cultured fibroblasts obtained from explants of normal and periodontally diseased human gingiva. Cells from normal tissues produce type I and III collagens in approximately the same proportion as they are found in most tissues. Fibroblasts derived from the diseased tissue appear to produce normal amounts of collagenous proteins, and type I and III collagens are made. However, in addition these cells synthesize a new collagen identified as type I trimer[2] (Narayanan & Page, 1976; Narayanan et al., 1978). Whether or not the type I trimer collagen is present in diseased tissues is not known. Nor is it known if additional collagens such as A and B, which were only recently described (Burgeson et al., 1976; Rhodes & Miller, 1978), occur in diseased tissues. In order to answer some of these questions, we have isolated and characterized the constituent collagens of diseased human gingiva.

Experimental Procedures

Materials

The gingival tissue used consisted of surgical specimens obtained from patients with chronic periodontitis of varying severity. Standard type I and III collagens were obtained from human fetal skin by methods described by Epstein (1974) and Chung & Miller (1974). Pepsin (sp act. 2500 units/mg) was the product of Worthington Biochemical Corp., Freehold, NJ. Ion-exchange celluloses were the products of Whatman Biochemicals Ltd., Maidstone, Kent, U.K. P-2, Bio-Gel A-5M (200–400 mesh) and the chemicals for electrophoresis were purchased from Bio-Rad Laboratories, Richmond, CA. All

† From the Department of Pathology SM-30, University of Washington, Seattle, Washington 98195. *Received May 6, 1980.* This work was supported by National Institutes of Health Grants DE-02600 and DE-03301.

[1] Because neither the stoichiometry of the A and B chains nor their relationship has been clearly resolved, we refer to these simply as A and B chains. These chains may originate from collagens of composition A_3, B_3, or AB_2 or from collagens containing additional chains designated as αC (Brown & Weiss, 1979; Sage & Bornstein, 1979).

[2] This term represents the homotrimer of α1[I] chains.

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other chemicals not listed above were of analytical grade and obtained from Mallinckrodt, Baker, or Fisher Chemical Co.

Methods

Extraction and Fractionation of Collagens. All preparations were carried out at 4 °C. The gingival tissue samples were pooled, minced, suspended in 10 volumes of 1 M NaCl in 0.05 M Tris,[3] pH 7.5, and homogenized by using a Polytron homogenizer (Brinkmann Instruments). After it had been stirred overnight at 4 °C, the extract was harvested by centrifugation and the residue reextracted 3 more times in a similar manner. These extracts were combined and the residue was extracted 5 times in 5 volumes of 0.5 M acetic acid and again 5 times in the same solution containing 1 mg/mL pepsin. The final residue was discarded and the pepsin extract subjected to NaCl fractionation. Collagens soluble in 1.5 and 2.5 M NaCl and insoluble in 1.5 M NaCl were obtained as described previously (Narayanan & Page, 1976).

$NaDodSO_4$[3]–Polyacrylamide Slab Gel Electrophoresis. Collagen α chains were separated electrophoretically on 5% gel slabs by using a 2.5% stacking gel as described by Laemmli (1970) and Studier (1973), with or without mercaptoethanol reduction, and the protein bands were located by staining with Coomassie Blue. Gels containing 3.6 M urea were used to separate α1[I] and α1[III] chains (Hayashi & Nagai, 1979). CNBr peptides were separated on 15% gel slabs with 5% stacking gels.

CM-cellulose Chromatography. Various collagen α chains were separated on a CM-cellulose column (0.9 × 9.0 cm) at 42 °C by using 0.02 M sodium acetate buffer, pH 4.8, containing 1 M urea with a NaCl gradient of 0–110 mM in a total volume of 200 mL of buffer (Narayanan & Page, 1976). The column was standardized with human types I, II, and III collagens. CNBr peptides were separated on a column (0.9 × 19 cm) at 42 °C with a gradient of 30–170 mM NaCl in 200 mL of 0.02 M sodium formate, pH 3.8 (Lichtenstein et al., 1975).

Phosphocellulose Chromatography. A and B chains were separated on phosphocellulose columns (0.9 × 9.0 cm) at 42 °C by using 0.03 M Na_2HPO_4, pH 6.3, with a NaCl gradient of 0–300 mM in a total volume of 200 mL of buffer (Rhodes & Miller, 1978).

Determination of Type I/III Ratios. Gingival tissue was homogenized in ~5 volumes of 70% formic acid with a Polytron homogenizer and digested with 10 times its weight of CNBr for 4 h at 37 °C with occasional shaking. The material was lyophilized and the peptides were separated by CM-cellulose chromatography as described above. Fractions containing CB8 peptide were pooled, desalted on a P-2 column, and then separated by gel filtration through a Bio-Gel agarose 5M column as described by Epstein (1974). The type I/III ratios were calculated by comparing the peak areas of respective CB8 peaks and correcting for their molecular weights (Epstein, 1974). Standards consisting of various ratios of type I and III collagens were also run for reference.

Amino Acid Analysis. Samples were hydrolyzed for 24, 48, or 72 h in constant boiling HCl at 108 °C, and the amino acids were separated and analyzed by using a Beckman 120C amino acid analyzer (Narayanan & Page, 1976).

Results

Extraction of the homogenized gingiva in 0.05 M Tris–1 M NaCl and in 0.5 M acetic acid removed between 7 and 17% of the total collagens present, but, as with other tissues, the major portion of the collagen remained insoluble. The five extractions with acetic acid containing 1 mg/mL pepsin solubilized almost all of the remaining collagen. The pepsin-solubilized collagen was separated into 2.5 and 1.5 M NaCl soluble and 1.5 M NaCl insoluble fractions as described under Methods. All the fractions were subjected to $NaDodSO_4$–polyacrylamide 5% slab gel electrophoresis in order to identify the various collagen α chains.

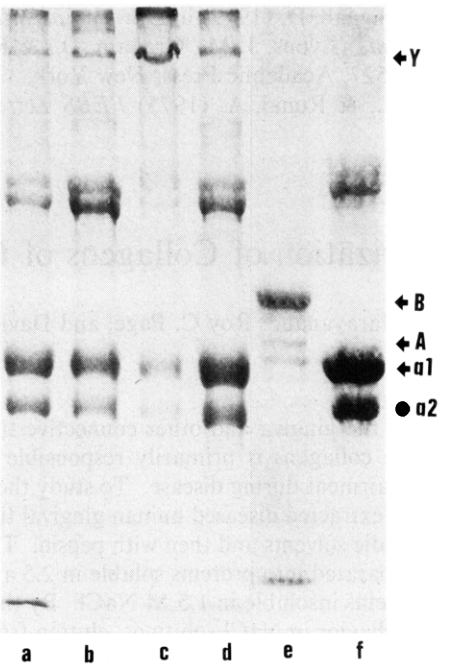

FIGURE 1: $NaDodSO_4$–polyacrylamide gel (5% slab) electrophoresis of collagen fractions of human gingiva. (a) NaCl extract; (b) acid extract; (c–e) pepsin extracts; (c) 1.5 M NaCl residue; (d) 1.5 M NaCl extract; (e) 2.5 M NaCl extract; (f) type I standard. The relative migration of α chains is indicated. Y indicates γ chains. The molecular weights of B and A chains are 125 000 and 108 000, respectively, based on collagen standards.

The 0.05 M Tris–1 M NaCl and acetic acid extracts contained predominantly α1 and α2 collagen chains plus cross-linked β and γ components, with the cross-linked chains present in greater proportions in the acid than in the salt extract (Figure 1a,b). The collagen in these fractions exhibited an α1 to α2 ratio of 2.0 and eluted from CM-cellulose columns in the positions of α1, α2, and β chains as expected of type I collagen (data not shown). Also reduction with mercaptoethanol did not affect the electrophoretic mobilities of these components. Thus, the 0.05 M Tris–1 M NaCl and acetic acid fractions contained predominantly type I collagen.[4]

The electrophoresis pattern of the 1.5 M NaCl insoluble fraction of the pepsin extract revealed small amounts of components migrating in the positions of α1 and α2 chains and a major component near the position of γ chains (Figure 1c). On CM-cellulose chromatography, most of the material eluted at the position of type III chains, but small peaks were

[3] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; CM, carboxymethyl; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

[4] Collagens of the acetic acid extracts could be separated into proteins that precipitate at 0.7 and at 1.2 M NaCl. The latter fraction represented <1% of acid-extracted collagens. $NaDodSO_4$–polyacrylamide gel (5% slabs) electrophoresis revealed that protein bands migrating with the A and B chains were present in this fraction. The mobilities of these bands were not significantly affected by pepsin digestion, indicating that they perhaps represent acid-extracted A and B chains. Thus it appears possible to obtain A and B chains, probably with intact telopeptides, by extracting with 0.5 M acetic acid. However, further characterization of these bands was not done because of insufficient material.

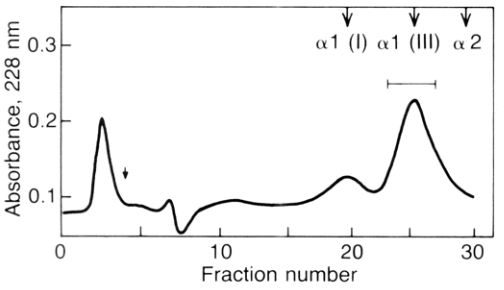

FIGURE 2: CM-cellulose chromatography of 1.5 M NaCl residue fraction of pepsin extract. Conditions of chromatography are given under Methods. A 5-mg sample was loaded, and fractions of 6.9 mL were collected. The elution of $\alpha 1$ and $\alpha 2$ chains is indicated on the basis of a run using standard type I and III collagen chains. Fractions pooled for further processing are shown.

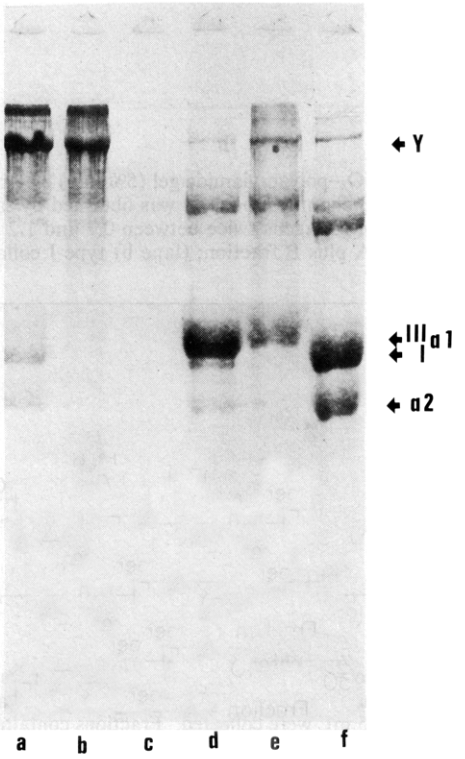

FIGURE 3: $NaDodSO_4$–polyacrylamide gel (5% slab) electrophoresis of type III fraction from Figure 2 before and after reduction in the presence of 3.6 M urea. (a) Skin type III, unreduced; (b) gingival type III, unreduced; (d) skin type III reduced; (e) gingival type III, reduced; (f) type I collagen. The migration of different $\alpha 1$ chains is indicated. Y indicates γ chains.

also present in the positions of $\alpha 1$[I] and $\alpha 2$ chains (Figure 2). Fractions under the type III peak were pooled as indicated (Figure 2), desalted, and subjected to electrophoresis with and without reduction in the presence of 3.6 M urea, a condition that will separate $\alpha 1$[I] and $\alpha 1$[III] chains (Hayashi & Nagai, 1979). As would be expected of type III collagen, the material migrated with γ chains before (Figure 3b) and with $\alpha 1$[III] chains after reduction (Figure 3e); thus, the 1.5 M NaCl insoluble fraction is predominantly type III collagen. To confirm this conclusion, we prepared cyanogen bromide peptides and analyzed them electrophoretically. The resulting pattern was identical with that of peptides prepared from type III collagen of human fetal skin (Figure 4). The type III chains purified by CM-cellulose chromatography were also subjected to amino acid analysis for comparison with type III collagen of human skin. As shown in Table I, the amino acid compositions of both were very similar. However, the gingival

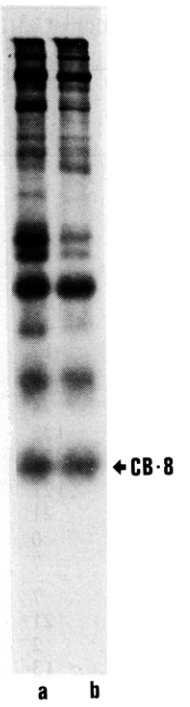

FIGURE 4: $NaDodSO_4$–polyacrylamide gel (15% slab) electrophoresis of CNBr peptides of type III collagen from (lane a) gingiva and (lane b) skin. The position of III–CB8 is indicated.

Table I: Amino Acid Composition of $\alpha 1$[III] Chains of Diseased Human Gingiva[a]

amino acid	residues/1000 human gingiva	residues/1000 human skin[b]
Hyl	14	5
Lys	29	30
His	6	6
Arg	49	46
3-Hyp	0	
4-Hyp	100	121
Asp	55	48
Thr	19	15
Ser	41	41
Glu	64	71
Pro	109	102
Gly	329	355
Ala	95	92
Val	19	16
$^1/_2$-Cys	2	2
Met[c]	7	7
Ile	20	13
Leu	26	21
Tyr	3	2
Phe	11	8

[a] The values are the mean of three determinations and were corrected for loss during hydrolysis. [b] From Epstein (1974). [c] Determined as methionine plus methionine sulfoxide and sulfone.

type III chains appeared to contain more hydroxylysine plus lysine and less hydroxyproline plus proline, and lysine was hydroxylated to a greater extent.

Electrophoresis patterns of the 1.5 M NaCl soluble fraction revealed the presence of $\alpha 1$ and $\alpha 2$ chains in a ratio of approximately 2:1, and in addition β and γ components were also present (Figure 1d). CM-cellulose chromatography and CNBr digestion showed that $\alpha 1$[I] and $\alpha 2$ chains were present in addition to small amounts of $\alpha 1$[III] chains (not shown).

Table II: Amino Acid Composition of α1[I] and α2 Chains of Diseased Human Gingiva[a]

amino acid	gingiva α1[I]	human skin α1[I][b]	gingiva α2	human skin α2[b]
Hyl	5 (5–14)	5	17	9
Lys	32	31	23	23
His	2	2	11	11
Arg	51	51	52	53
3-Hyp	0	1	0	1
4-Hyp	82	101	79	86
Asp	42	43	42	46
Thr	17	17	21	19
Ser	36	39	28	36
Glu	70	74	73	69
Pro	132	139	118	123
Gly	351	352	333	350
Ala	117	121	105	113
Val	21	21	37	34
½-Cys	0	0	0	0
Met[c]	7	7	9	5
Ile	7	7	15	15
Leu	18	21	26	33
Tyr	1	2	2	4
Phe	13	13	11	12
total	1004	1047	1002	1042

[a] The values are the mean of three determinations and were corrected for loss during hydrolysis. [b] From Epstein et al. (1971). [c] Determined as methionine plus methionine sulfoxide and sulfone.

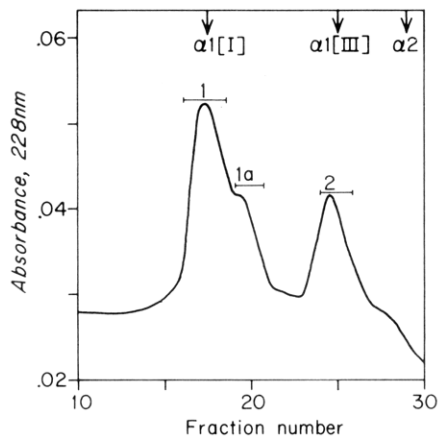

FIGURE 5: CM-cellulose chromatography of 2.5 M NaCl soluble collagens of the pepsin extract. Conditions were the same as those described for Figure 2. Fractions were pooled as shown, desalted, and then processed for amino acid composition.

Thus, this fraction contained predominantly type I collagen. The amino acid composition of α1 and α2 chains purified by CM-cellulose chromatography is presented in Table II. The composition was virtually identical with the respective chains from human skin.

Electrophoresis patterns of the 2.5 M NaCl soluble fraction revealed protein bands in the region of α chains exhibiting molecular weights of 100 000, 108 000, and 125 000 based on collagen standards (Figure 1e). The components with M_r 108 000 and 125 000 were not pro α chains, because they were not converted to α chains by redigesting the fraction with pepsin (not shown). Therefore, these appeared to be additional collagen components not previously detected in the human gingival tissue. The presence of α1 chains (M_r 100 000) and the absence of α2 chains indicated that this fraction also contained a collagen consisting of α1 chains. In order to further establish the identity of these components, we subjected the 2.5 M NaCl soluble fraction to CM-cellulose chromatography. As seen in Figure 5, a major peak with a shoulder eluted in the position of standard α1[I] (peaks 1 and 1a) and another near type III chains (peak 2). Very little material was found in the position of α2 chains. Fractions under each peak were pooled and subjected to amino acid analysis. Peaks 1 and 1a exhibited an amino acid composition similar to that of α1[I] chains but with minor differences (Table III). The composition of peak 2 differed significantly from that of α1-[III] and α1[I] in having notably higher hydroxylysine and lower alanine contents. These are features of A and B chains (Rhodes & Miller, 1978). Therefore, in order to further separate these components, we dissolved the 2.5 M NaCl soluble material in 0.5 M acetic acid at 4 °C and harvested the material that precipitated between 0.7 and 1.2 M NaCl (Rhodes & Miller 1978). The collagens obtained were re-purified once again in a similar fashion. $NaDodSO_4$–polyacrylamide gel electrophoresis revealed that this material comprised protein bands corresponding to A and B chains (Figure 6a). Chromatography on a phosphocellulose column resolved the fraction into two major and one minor peaks

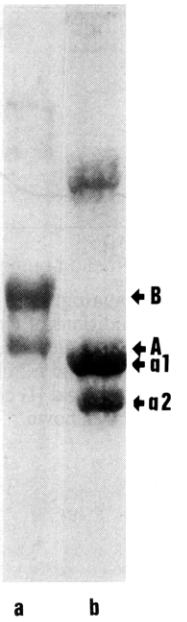

FIGURE 6: $NaDodSO_4$–polyacrylamide gel (5% slab) electrophoresis of gingival A and B chain fraction. It was obtained by salting out 2.5 M NaCl soluble collagens twice between 0.7 and 1.2 M NaCl. (Lane a) Purified A plus B fraction; (lane b) type I collagen.

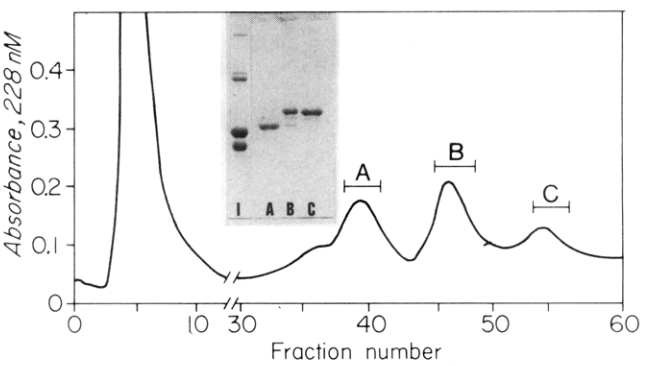

FIGURE 7: Phosphocellulose chromatography of A plus B chain fraction. Conditions are given under Methods. 4.5 mg was loaded, and fractions of 3.45 mL were collected. Fractions containing peaks A, B, and C were pooled as shown, purified by rechromatography, and then further characterized. (Insert) $NaDodSO_4$–polyacrylamide slab gel (5%) electrophoresis of type I collagen (lane 1) and peaks A, B, and C.

Table III: Amino Acid Composition of CM-cellulose Chromatography Peaks (Figure 5) of 2.5 M NaCl Soluble Collagens[a]

amino acid	residues/1000		
	peak 1	peak 1a	peak 2
Hyl	8	8	26
Lys	24	34	22
His	2	3	8
Arg	40	36	39
3-Hyp	0	0	0
4-Hyp	69	75	78
Asp	55	52	57
Thr	23	23	24
Ser	53	50	46
Glu	85	86	96
Pro	112	125	104
Gly	332	300	339
Ala	120	117	73
Val	23	24	24
Ileu	9	16	15
Leu	23	26	33
$^1/_2$-Cys	0	0	0
Met[b]	2	5	4
Tyr	4	4	2
Phe	16	16	9

[a] Values were not corrected for loss during hydrolysis. [b] Determined as methionine plus methionine sulfoxide and sulfone.

Table IV: Amino Acid Composition of Phosphocellulose Chromatography Peaks A, B, and C (Figure 7)

amino acid	residues/1000				
	peak A[a]	A chains[b]	peak B[a]	B chains[b]	peak C[c]
Hyl	22 (20–27)	23	34	36	32
Lys	14	13	18	14	21
His	11	10	7	6	10
Arg	47	48	38	40	34
3-Hyp	0	3	0	5	0
4-Hyp	110	106	106	110	96
Asp	52	50	50	49	54
Thr	28	29	23	21	22
Ser	40	34	31	23	40
Glu	81	89	95	100	99
Pro	97	107	132	130	106
Gly	341	331	324	332	322
Ala	54	54	44	39	61
Val	26	27	20	17	22
$^1/_2$-Cys	0		0		3
Met[d]	12	11	9	9	6
Ile	15	15	18	17	17
Leu	36	37	37	36	39
Tyr	2	2	3	4	4
Phe	11	11	11	12	13

[a] The values represent the mean of six determinations and were corrected for loss of threonine, serine, and tyrosine during hydrolysis. [b] From Rhodes & Miller (1978). [c] One determination. [d] Determined as methionine plus its sulfoxide and sulfone.

(Figure 7, peaks A–C). Peaks A and B, respectively, possessed electrophoretic migration corresponding to A and B chains, and peak C migrated somewhat slower than B chains[5] (Figure 7, inset). Peaks A and B were rechromatographed, and their amino acid compositions were determined. Their compositions were very similar to those of A and B chains from human placenta (Table IV) and only minor differences were detected.

[5] For chromatographically purified peaks A, B, and C, M_r values of 106 000, 126 000, and 123 000 were obtained from collagen standards. However peaks B and C could not be resolved into two separate bands by electrophoresis of fractions containing all three components.

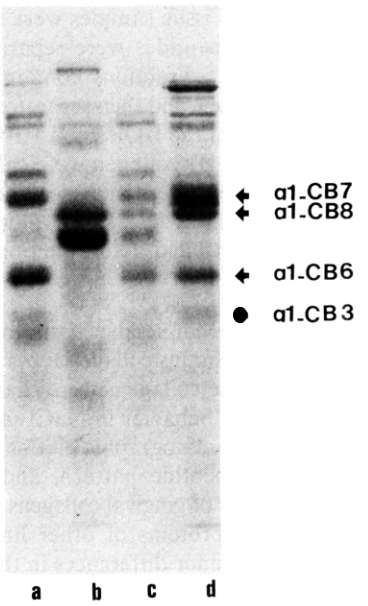

FIGURE 8: $NaDodSO_4$–polyacrylamide gel (15% slab) electrophoresis of CNBr peptides of A and B peaks of Figure 7. (Lane a) Peak B; (lane b) peak A; (lane c) mixture of A and B; (lane d) α1[I] chain. The type I CB peptides have been marked.

Table V: Proportion of Various Collagens Extractable from Inflamed Human Gingiva[a]

collagen type	weight yield	
	mg	%
I	119.3	86.8
III	5.2	3.8
type I trimer	2.0	1.5
types A plus B[b]	11.0	8.0
total	137.5	100.1

[a] 7.93 g of inflamed human gingiva obtained from periodontal surgery was extracted, and the collagens were separated by NaCl fractionation, as described under Methods. [b] This fraction was obtained after repurification of the 2.5 M NaCl fraction by salting out between 0.7 and 1.2 M NaCl as described under Results. By electrophoresis B/A ratio was 3:1, therefore this fraction has 2.8 mg (2.0% of total) of A and 8.3 mg (6.0% of total) of B chains.

The only notable difference was the lack of 3-hydroxyproline in the gingival A and B chains. The identity of these chains was further confirmed by analysis of the CNBr peptides. As shown in Figure 8, the peptide patterns for the A and B peaks differed one from the other (Figure 8) and from the patterns for α1[I] and α1[III] chains, although they closely resembled respectively the patterns described for A and B chains of human fetal membranes by Burgeson et al. (1976) and Sage & Bornstein (1979). The composition of peak C differed significantly from that of A and B chains, notably in having cysteine (Table IV). Its identity could not be pursued by further characterization because of low recovery of the material.

The proportions of various collagens obtained in a typical preparation, based on weight yields, are presented in Table V. Type I collagen was the predominant component making up 87% of the total extracted collagens, with A plus B following with 8%. The type III and type I trimer accounted for 4% and <2% of the total, respectively. It is likely that the recovery of type III collagen in our experiments was low because it was destroyed by pepsin during the extraction process (Burke et al., 1977). To exclude this possibility, we determined the proportion of type III collagen by the CNBr digestion

method (Epstein, 1974). Tissue samples were digested with CNBr, and the resulting peptides were separated by CM-cellulose chromatography. Fractions containing the CB8 peptides were pooled, desalted, and then separated on a column of Bio-Gel A-5M, and from the I-CB8 and III-CB8 peptides obtained, the ratios of I to III collagen were calculated (Epstein, 1974). In three independent analyses, the type I/type III ratios were 97:3, 96:4, and 94:6. These values are in good agreement with the weight yield determination in Table V.

Discussion

Our data show that the connective tissue of chronically inflamed human gingiva contains collagen types I, III, A, and B, and type I trimer. These collagens were characterized on the basis of their solubility behavior in NaCl solutions, electrophoretic migration, chromatographic elution from ion-exchange columns, CNBr peptide pattern, and amino acid composition. The properties of gingival collagens are in general similar to the respective proteins of other human tissues. However, there are some minor differences in the amino acid composition. For instance, the gingival α chains possess greater amounts of lysines (lysine plus hydroxylysine) and lesser amounts of prolines (proline plus 4-hydroxyproline). The lysine hydroxylation of gingival collagens was greater. We were not able to detect 3-hydroxyproline in any of the α chains even by overloading the amino acid analyzer columns. The reasons for these differences are not clear; however, the close resemblance of CNBr peptide patterns to respective collagens from other tissues indicates that these differences perhaps represent tissue to tissue variations in the postribosomal modification reactions rather than different gene products.

We noticed that the B/A ratios in different preparations of initial 2.5 M NaCl soluble collagens were high and about 5:1. However, after repurification by salting out between 0.7 and 1.2 M NaCl, ratios of 2:1 were consistently obtained (for instance, compare Figure 1e with Figure 6a and Table V). Although several factors may account for this phenomenon, one reason may be the presence in the initial fractions of additional chains, such as peak C, that possess electrophoretic mobilities similar to B chains and which are removed by repurification. Alternatively some pepsin may remain bound and be active on chains, thereby causing erroneous ratios. Thus, from our experiments it is not possible to determine whether in the gingiva the A and B chains represent separate homotrimers (Rhodes & Miller, 1978; Deyl et al., 1979) or heteropolymers composed of A and B (Burgeson et al., 1976; Welsh et al., 1980) and perhaps additional chains (Brown & Weiss 1979; Sage & Bornstein, 1979).

The peak C material (Figure 7) is less than B chains in molecular size by 3000 daltons, and it is different in its composition, notably in having cysteine and more alanines. The composition is also different from that of "C" chains of human fetal membranes (Sage & Bornstein, 1979; Kresina & Miller, 1979) and bovine tissues (Brown & Weiss, 1979). It does not appear to be pro α chains by its composition and resistance to pepsin. Therefore it may be a distinct α chain; however, due to the lack of further characterization data, it is not clear whether it represents a separate collagen or whether it is a constituent of a collagen that has A and/or B chains in addition.

The 2.5 M NaCl soluble fractions contain an α1 chain that resembles α1[I] in electrophoretic and ionic properties. The amino acid composition is also similar, except for a slightly greater hydroxylation of lysines than the latter. It is not a degradation product of A or B chains, because their amino acid compositions are different. From these data and from the lack of α2 chains, it appears that this α1 chain represents a collagen of composition $\alpha 1[I]_3$. In similar studies Uitto (1979) has observed that normal human skin contains 4% of its collagens as type I trimer. The type I trimers of skin tissue and amniotic fluid cells also have their lysines hydroxylated to a greater extent than α1[I] chains (Uitto, 1979; Crouch & Bornstein, 1978). The detection of type I trimer collagen in the diseased human tissue is in accordance with the previous observations with fibroblast cultures. However in the diseased gingiva, in contrast to cell cultures and skin tissue (Uitto, 1979), the type I trimer was present in lower proportions. One reason for this may be that the type I trimer is more susceptible to degradation by enzymes present in the inflamed tissue than are other collagens and it may turn over more rapidly. Alternatively, because of environmental conditions, the cells in vivo may synthesize less type I trimer than do cells maintained in culture.

In the gingiva, as in most other connective tissues except cartilage, type I is the major collagen component. The type III collagen accounts for <5% of the total, whereas in other connective tissues it is the second major collagen fraction. This is not due to the loss by degradation of type III collagen by pepsin which was used for the extraction (Burke et al., 1977), because CNBr digestion of whole tissue followed by quantitation based on relative ratios of I-CB8/III-CB8 peptides gave the same results. Unexpectedly, the A and B chains together were present in quantities greater than type III, and they accounted for 8% of the total collagens. Thus the diseased gingival connective tissue appears to be unique in its collagen composition. It is also different from other oral tissues; for instance, in edentulous ridge tissue which replaces gingiva after tooth loss, type III and A plus B chains account for 15% and <1% of the total collagens, respectively (Narayanan et al., 1980).

The disease state is likely to be an important determinant of the collagenous composition of inflamed gingiva. The various collagens differ in their susceptibility to enzymatic degradation, and the diseased tissue abounds in a variety of degradative enzymes originating from neutrophils and macrophages. The greater susceptibility of type III collagen (Miller et al., 1976; Burke et al., 1977) and the resistance of A and B chains to these enzymes (Liotta et al., 1979; Sage & Bornstein, 1979) may account, in major part, for the relatively large A plus B and the unexpectedly small type III components. Similar degradative processes may also account for the small amount of type I trimer in the diseased tissue relative to the amount found in cell cultures.

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Conformational Dynamics of Insulin in Solution. Circular Dichroic Studies†

Y. Pocker* and Subhasis B. Biswas

ABSTRACT: Conformational changes of bovine insulin in solution with concentration and pH detected by circular dichroic (CD) studies are reported. The change in the CD spectrum of insulin in the higher concentration range (from 100 μM down to 2 μM) is relatively small, but in the lower concentration range (from 2 μM down to 60 nM) the CD spectrum changes substantially with concentration. A detailed analysis of the data indicates that the hormone has two major conformational states: conformation I, a form which predominates in extremely dilute solutions and corresponds to the monomeric state, and conformation II, a form present in the crystalline state and also, with but minor changes, in all associated states in solution. The apparent conformation of insulin at various concentrations is computed by using a nonlinear least-squares iterative computer program. The mean residue ellipticities at 223 and 208 nm are extrapolated by using $[\theta]_\lambda$ vs. $f_{monomer}$ plots to calculate the conformations of monomeric and dimeric insulin. These calculations indicate that conformation I of insulin has 21% less helix content than conformation II, the latter conformation being very similar to that found in the crystalline state. It is also evident from these calculations that the conformational transition is of the helix–coil type. Studies pertaining to the dependence of the CD spectrum of insulin on pH are also reported, and a comparison is made with earlier sedimentation coefficient studies. An analysis of the data indicates that changes in the sedimentation coefficient correspond quite closely to changes of CD spectra with pH. This paper is the first report known to us pertaining to conformational studies of insulin in the monomeric state; it presents evidence for conformational transitions of the protein hormone induced by concentration and pH. Since insulin is biologically active mainly in the monomeric state, a knowledge of its conformation in this state should be an important tool in deciphering the molecular basis of insulin action.

At the present time the effects of insulin in biological systems are reasonably well-known, but the mechanism of action is still not well understood. Recent studies indicate that the first step in the action of insulin is its binding to a receptor site on the cell membrane (Cuatrecasas, 1974; Freychet, 1976; Kahn, 1975; Roth et al., 1975). This binding alters the transmembrane transport processes, and the dynamics of binding in turn depend on the conformation of protein hormone in solution.

In the last few years, several groups have investigated the conformation of insulin in the crystalline form and in solution (Blundell et al., 1971; Dodson et al., 1979; Goldman & Carpenter, 1974; Peking Insulin Structure Research Group, 1974; Wood et al., 1975). Through these studies, the structure of insulin in the crystalline state is now known at a resolution of 1.5 Å. The conformation of insulin in the solution is still not fully delineated.

Insulin exhibits a complex association behavior in crystal and in solution. In the crystalline state it exists as a hexamer and in solution it exists as an equilibrium mixture of monomers, dimers, tetramers, hexamers, and possibly some higher associated states. The studies on the association behavior of insulin by equilibrium sedimentation (Goldman & Carpenter, 1974; Jeffrey & Coates, 1965, 1966; Pekar & Frank, 1972) indicate that under physiological conditions this protein hormone must exist exclusively as a monomer. So far, the conformation of insulin in the monomeric state has not been studied and, therefore, is totally unknown.

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