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Band Pattern of the Segment-Long-Spacing Form of Collagen. Its Use in the Analysis of Primary Structure†

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ABSTRACT: The highly reproducible banded pattern of segment-long-spacing crystallites observed in the electron microscope has been characterized in terms of the number and location of bands in order to facilitate its use in the primary structure analysis of collagen. Positively stained crystallites of calf skin collagen show 117 characteristic dark and light bands distributed in an aperiodic pattern. The location in the pattern of individual bands, designated by simple numbers, has been determined by an optical averaging procedure. The data enable us to define and report the precise location of individual bands and segment-long-spacing fragments along the length of the molecule. Since the segment-long-spacing band pattern of calf skin collagen represents the pattern of crystallites prepared from a wide variety of collagens, it also has been possible to collocate, on a standard basis, a large number of published observations on segment-long-spacing fragments produced by enzymatic or specific chemical cleavage, and the most recent data correlating particular bands with specific amino acid sequences.

Electron microscopy of segment-long-spacing crystallites has played a significant role in both the amino acid sequence analysis of collagen and in studies on the specific sites of cleavage by various collagenases (Piez *et al.*, 1968; Rauterberg and Kuhn, 1968; Stark and Kuhn, 1968a; Fietzek *et al.*, 1970; Igarashi *et al.*, 1970; Gross and Nagai, 1965; Rauterberg *et al.*, 1970; Harper *et al.*, 1971). These applications of electron microscopy to the study of molecular structure are possible because of the unusual properties of the collagen molecule. The long (3000 × 14 Å), rigid, triple-helical molecules, in the presence of adenosinetriphosphoric acid (ATP) at low pH (Schmitt *et al.*, 1953; Gross *et al.*, 1954), align in perfect transverse register to form crystallites, called *segment-long-spacings*, that are the same length as the collagen molecule. When stained with solutions of phosphotungstic acid or uranyl acetate, such crystallites reveal a characteristic band pattern which reflects the distribution of clusters of charged and uncharged amino acids along the molecule (Hodge and Schmitt, 1960; Mark *et al.*, 1970b; Balian *et al.*, 1971); thus individual bands of a segment-long-spacing crystallite serve to identify loci (*ca.* 20 Å) on the collagen molecule.

In the amino acid sequence analysis of collagen, the linear order of large peptide fragments has been established by relating the fragments to particular groups of bands in the segment-long-spacing band pattern, *e.g.*, cyanogen bromide (CNBr) peptides, prepared from isolated α chains, re-form triple-helical structures upon temperature annealing (Rauterberg and Kuhn, 1968), and when allowed to react with ATP such structures form fragments of segment-long-spacing crystallites that have all the detailed banding characteristic of their region of origin in the native structure. To date it has been possible to renature and order by electron microscopy four of the nine CNBr peptides of the α1 chain and three of the five peptides of the α2 chain, which range in molecular weight from 13,500 to 30,000 (Rauterberg and Kuhn, 1968; Fietzek *et al.*, 1970; Igarashi *et al.*, 1970). By combining electron microscopic data on the larger fragments with biochemical data on the smaller ones, it has been possible to establish the proper order of all the cyanogen bromide peptides in the α1 and α2 chains of several mammalian collagens (Piez *et al.*, 1968, 1969; Vuust *et al.*, 1970).

The segment-long-spacing band pattern also has been used to locate sites of cleavage by specific enzymatic or chemical methods (Gross and Nagai, 1965; Stark and Kuhn, 1968b; Heidemann and Heinrich, 1970; Hörmann and Volpin, 1970; Nordwig and Bretschneider, 1971) and to visualize directly, by specific staining, bands containing particular reactive residues such as arginine or methionine (Weiss and Bowden, 1969a,b).

A serious limitation in all of these studies has been the absence of a quantitative characterization of the segment-long-spacing band pattern in terms of the number and location of bands. In this paper we establish the number and position of reproducible bands in segment-long-spacing crystallites of calf skin collagen and demonstrate the application of this information to the structural analysis of collagen molecules.

Materials and Methods

Collagen. Purified acid-soluble calf skin collagen was prepared according to the method of Gross and Kirk (1958), lyophylized, and stored frozen until used. The collagen was solubilized in 0.5 M acetic acid (4°) at a concentration of 0.1%;

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insoluble material and aggregated forms were removed by centrifugation at 105,000*g* for 60 min (4°).

Segment-Long-Spacing Crystallites. About 0.25 ml of the collagen solution was dialyzed overnight at 4° against 3 ml of 0.4% sodium adenosine 5′-triphosphate or adenosine-5′-triphosphoric acid (K&K Laboratories) in 0.1 M acetic acid (Stark and Kuhn, 1968a). The resultant suspension of segment-long-spacing crystallites was diluted to a faint cloudiness with the ATP solution.

Electron Microscopy. Drops of the segment-long-spacing crystallite suspension were applied to 400 mesh specimen grids covered with collodion films lightly coated with carbon. After 5 min the drops were removed with filter paper; the preparations were stained for 3 min with 0.1% phosphotungstic acid, rinsed very briefly with water, dried, stained for 5 min with 0.01% uranyl acetate, rinsed again with water, and dried (Stark and Kuhn, 1968a). The entire procedure was carried out at 4° or at ice-bath temperature.

The preparations were examined with a JEOL JEM-100B electron microscope operated at 60 kV with a 20-μ objective aperture and a decontamination device cooled with liquid nitrogen. The magnification of the microscope was determined repeatedly with a cross-lined replica of a diffraction grating having 2160 lines/mm or with crystallites of myosin (phase B) having a regular repeating period of 143 Å (King and Young, 1972). Micrographs of the segment-long-spacing crystallites were made on Kodak Contrast Lantern Slide Plates at a magnification of 50,000–70,000×.

Optical Averaging of the Segment-Long-Spacing Band Pattern. On first observation of positively stained segment-long-spacing crystallites, the band pattern appears to consist of a series of parallel, alternating, dark and light lines. On closer examination at high resolution, however, each dark band consists of numerous irregular densities scattered along lines which usually are not straight. Such irregularities must be taken into account to establish accurately the linear distances from band to band. Accordingly, we have "averaged" the location of such irregularities with the following optical procedure. The image of a narrow strip of the complete segment-long-spacing band pattern (*ca.* 5 mm wide at a magnification of *ca.* 500,000×) is projected from an ordinary photographic enlarger onto photographic paper moving linearly, in a direction parallel to the bands, at a rate of *ca.* 3 cm/min. The resultant photograph consists of a series of straight lines of various widths and densities. The center of the most dense part of each line represents the weighted average position of the scattered densities that constitute the corresponding dark band in the original electron micrograph. True bands on the integrated image are distinguished from spurious lines by comparing averaged images from different crystallites. This method of optical averaging is a modification of a procedure used by Labaw (1971) and by Labaw and Davies (1971) for clarifying electron micrographs of γG1-globulin crystals. An equivalent method of optical averaging, which employs a cylindrical lens, has been described by Dowell *et al.* (1968).

Results

General Characteristics of the Segment-Long-Spacing Band Pattern. Figure 1a shows the 117 reproducible stained bands and unstained interbands observed in segment-long-spacing crystallites of acid-soluble calf skin collagen. The individual dark bands, identified by numbers, have been observed repeatedly in electron micrographs of well-formed crystallites. Although a total of 58 distinct dark bands have been identified, all of them are not resolved regularly in every crystallite; frequently adjacent dark bands are fused and appear as a single line; occasionally single dark bands appear to be resolved into two narrower bands (*e.g.*, band 23); and sometimes faint lines, which suggest the presence of additional bands, appear in interband regions. Such observations have been disregarded for the present because of the variabilities involved, and only those bands observed repeatedly in many micrographs have been numbered.

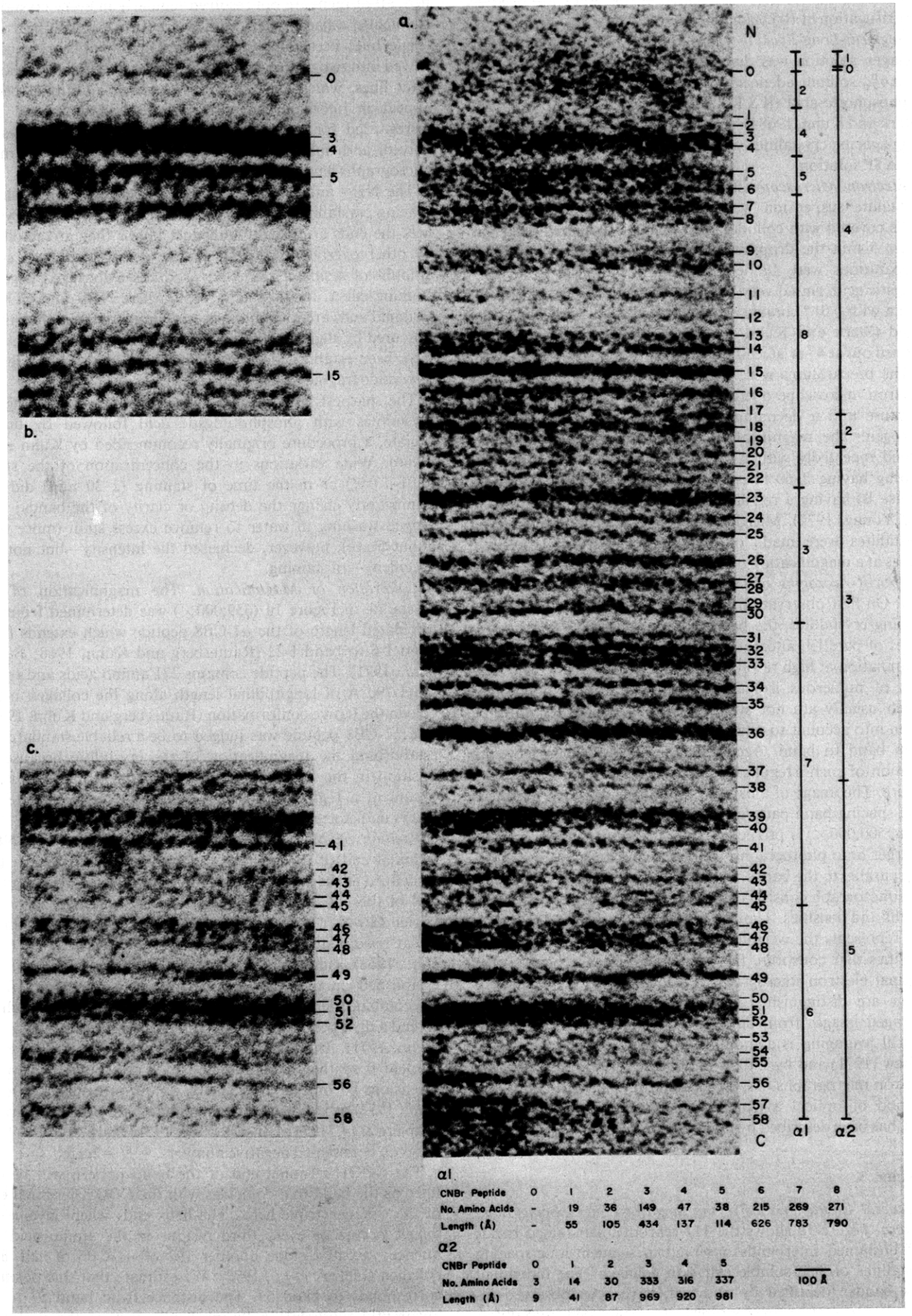
The NH_2- and COOH-terminal ends of the segment-long-spacing crystallites are seldom well defined. In single crystallites the ends are usually indistinct unless they are stabilized by other overlying segments or are demarcated by a small amount of residual stain that produces a localized negative staining effect. In polymeric forms where NH_2- and COOH-terminal ends are joined, the precise limits of the segments are obscured by slight overlapping. The exact location of the ends have been established, however, by using data on the length of peptide fragments (*vide infra*).

The sharpest band patterns were obtained by staining the crystallites with phosphotungstic acid followed by uranyl acetate, a procedure originally recommended by Kuhn *et al.* (1966). Wide variations in the concentration of the stains (0.01–1.0%) or in the time of staining (2–30 min) did not significantly change the density or clarity of the bands; prolonged washing in water to remove excess stain (more than about 5 sec), however, decreased the intensity—but not the specificity—of staining.

Calibration of Magnification. The magnification of the crystallite in Figure 1a (659,000×) was determined from the calculated length of the α1-CB8 peptide which extends from band I-6 to band I-22 (Rauterberg and Kuhn, 1968; Balian *et al.*, 1971). The peptide contains 271 amino acids and represents 790 Å of longitudinal length along the collagen molecule in the native conformation (Rauterberg and Kuhn, 1971). The α1-CB8 peptide was judged to be a reliable standard for establishing the magnification of the crystallite because its position in the band pattern is well defined and because it occurs in a region of the crystallite that most consistently shows undistorted bands.

Position of NH_2- and COOH-Terminal Ends. The NH_2-terminal end of the segment-long-spacing band pattern will be defined as the position that coincides with the NH_2-terminal end of the collagen triple helix. The helix begins where the triplet Gly-X-Y first appears in the amino acid sequence, *i.e.* at residue number 13 of the 15 residues of α1-CB1 (Piez *et al.*, 1968). This position (marked 0 in Figure 1a and 1b) was established on the segment-long-spacing band pattern from the combined length of the α1-CB peptides 2, 4, and 5, which extend a distance of 356 Å from interband I-6 (Rauterberg and Kuhn, 1971). The NH_2-terminal end of the pattern has been marked 0 so that if additional bands are found in peptide extensions beyond the triple helix, *e.g.*, in procollagen (Dehm *et al.*, 1972) or in collagen from animals with dermatosparaxis (Lapière *et al.*, 1971; Lenaers *et al.*, 1971; Stark *et al.*, 1971a), they can be assigned negative numbers, −1, −2, etc.

The COOH-terminal end of the band pattern will be defined as the band that coincides with the COOH-terminal end of the collagen triple helix. The helix ends where glycine no longer occurs as every third residue in the amino acid sequence, *i.e.*, at residue number 192 of α1-CB6 of calf skin collagen (Fietzek *et al.*, 1972). We estimate that this position corresponds to band 57. The distance from band 57 to 58 apparently represents a "nonhelical" part of the molecule which can be removed by the action of carboxypeptidase or


a.
b.
c.
N
C
α1
α2
100 Å
α1
CNBr Peptide 0 1 2 3 4 5 6 7 8
No. Amino Acids - 19 36 149 47 38 215 269 271
Length (Å) - 55 105 434 137 114 626 783 790
α2
CNBr Peptide 0 1 2 3 4 5
No. Amino Acids 3 14 30 333 316 337
Length (Å) 9 41 87 969 920 981

pepsin (Leibovich and Weiss, 1970) or chymotrypsin (Stark *et al.*, 1971b).

On the basis of the calculated magnification of the crystallite in Figure 1a and the estimated position of the NH_2- and COOH-terminal ends of the crystallite, the overall length of the band pattern (0–57) measures 2947 Å, a figure that agrees within 2% with the molecular length determined by light scattering and hydrodynamic measurements (Boedtker and Doty, 1956), and within 3% of the length calculated from the amino acid content of the α1 chain (Rauterberg and Kuhn, 1971).

Localization of Individual Bands. The location of each band and interband in Figure 1a was determined by optical averaging on longitudinal strips of electron micrographs from three different segment-long-spacing crystallites (Figure 2). The distance of each band from the NH_2-terminal end of the band pattern is recorded in Table I.

Systematization of Published Observations on Segment-Long-Spacing Crystallites. Since segment-long-spacing crystallites prepared from a wide variety of tissues and animals have a similar band pattern (*vide infra*), it has been possible to collocate, on the basis of the band numbers shown in Figure 1a, more than 100 published observations on positively stained segment-long-spacing crystallites. Selected examples from the comprehensive tabulation are shown in Table II (see footnote *a*). The observations include localization on the segment-long-spacing band pattern of: cyanogen bromide and hydroxylamine peptides, fragments produced by enzymatic cleavage, sites of scission by specific animal collagenases, amino acid residues identified by selective staining procedures, and clusters of specific residues where the amino acid sequence has been established.

Discussion

Similarity of Segment-Long-Spacing Band Patterns. Collagens from a wide variety of sources form segment-long-spacing crystallites with remarkably similar band patterns (Tkocz and Kuhn, 1969; Nordwig and Hayduk, 1969), an observation that can be confirmed by comparing published micrographs of collagen crystallites from: calf skin (Rauterberg and Kuhn, 1971); rattail tendon (Olsen, 1967a); cod fish skin (Olsen, 1967b); chick skin (Igarashi *et al.*, 1970); human skin (Leibovich and Weiss, 1971); pig skin (Heinrich *et al.*, 1971); guinea pig skin (Jeffrey and Gross, 1970; Donoff *et al.*, 1971); fish swim bladder and several invertebrates (Nordwig and Hayduk, 1969); and renatured α1 and α2 chains of calf skin and rat skin collagen (Tkocz and Kuhn, 1969). Moreover, we have confirmed the uniformity of the band pattern by preparing and examining segment-long-spacings of collagen from guinea pig, rat, and chick skin, rattail tendon, and carp swim bladder tunic. Although minor variations in the number, location, and staining intensity of bands may exist in these crystallites, the band pattern shown in Figure 1a is generally representative of these collagens.

Applicability of the Data. Heretofore the band pattern of segment-long-spacing crystallites has been used primarily in a qualitative manner for the study of collagen structure: electron micrographs of crystallite fragments have been matched to micrographs of the complete segment-long-spacing crystallite; attention has been directed to particular segments of the band pattern or to individual bands with lines, arrows, or other special symbols; and the observations have been discussed in terms of such eclectic designations or by awkward descriptive terms. The following examples illustrate how our characterization of the segment-long-spacing band pattern (Figure 1a, Table I) will permit precise identification of individual bands or segments of the band pattern and rapid communication of such information on the basis of simple numbers.

(1) When segment-long-spacing crystallites are used to identify and order peptide fragments, the position of a fragment can be expressed in terms of band numbers. Figure 1a and Table II, item 1, show that α1-CB8 occupies the segment from interband I-6 to I-22; α2-CB3 extends from interband I-19 to I-37, etc. The location of peptide fragments resulting from hydroxylamine cleavage or the position of triple-stranded fragments resulting from the degradation of native collagen molecules by bacterial collagenase also can be defined with band numbers (Table II, items 5, 6, and 11). As a corollary, the type of bond cleaved can be related to specific bands: methionine residues must occur at interbands I-6, I-22, etc. Finally, from the location of a peptide fragment on the band pattern and the data in Table I, it is a simple matter to determine the length of a fragment and hence to approximate its molecular weight. This systematization of the segment-long-spacing band pattern will be useful in the primary structural analysis of collagen from cartilage and other new sources (*vide infra*).

(2) In well-defined segments of the segment-long-spacing band pattern where amino acid sequences are known, stained bands have been related to clusters of charged amino acid residues distributed along the α1-chain (Table II, items 13–18). Such relations now can be quickly compared and discussed in terms of band numbers rather than on the basis of pictorial representations or descriptive terms, *e.g.*, the cluster of charged amino acid residues in positions 62–68 of peptide fragment α1* (780)-CBC corresponds to band number 49 (Table II, items 12, 13); residues 8–11 in the α1-CB8-HA1 fragment correspond to band number 7 (Table II, items 5 and 15). The correspondence between bands and amino acid sequences is sufficiently precise so that in sequencing operations it is sometimes possible to locate small sequenced peptides by matching the distribution of charged and uncharged

FIGURE 1: (a) Electron micrograph of a segment-long-spacing crystallite, prepared from native, acid-soluble calf skin collagen, showing the location of 117 characteristic bands and interbands. The dark *bands* (stained) are numbered consecutively from the NH_2-terminal (N) to the COOH-terminal (C) end of the band pattern. The light intermediate bands, or *interbands* (unstained and unnumbered), are designated according to the dark band they follow, *e.g.*, the light band between dark bands 25 and 26 is designated *interband twenty-five* or *I-25*. The distance of each band from the NH_2-terminal end of the band pattern is listed in Table I. The bands appear clearest to the eye when the micrograph is viewed at a low angle parallel to the bands. In the terminology of Hodge and Schmitt (1960), bands δ_1, δ_2, δ_3, and δ_4 correspond to bands 49, 36, 23, and 9–10, respectively. The schematic diagrams of α1 and α2 chains illustrate the relation of individual cyanogen bromide peptides of calf skin collagen to bands of the segment-long-spacing crystallite. The length of each peptide (table below micrograph) has been calculated from its amino acid content by Rauterberg and Kuhn (1971) and Fietzek *et al.* (1970); the linear order of the peptides has been determined by Piez *et al.* (1968, 1969), Rauterberg *et al.* (1970), Fietzek *et al.* (1970), and Igarashi *et al.* (1970). The amino acid content of the individual α2-CB3 and α2-CB5 peptides is taken from Kang *et al.* (1969). (b) Electron micrograph showing the NH_2-terminal end (0) of the band pattern. (c) Electron micrograph of the COOH-terminal region of the pattern showing several bands that are unresolved in part a. The specimens were stained with 0.1% phosphotungstic acid followed by 0.01% uranyl acetate; magnification, 659,000×.

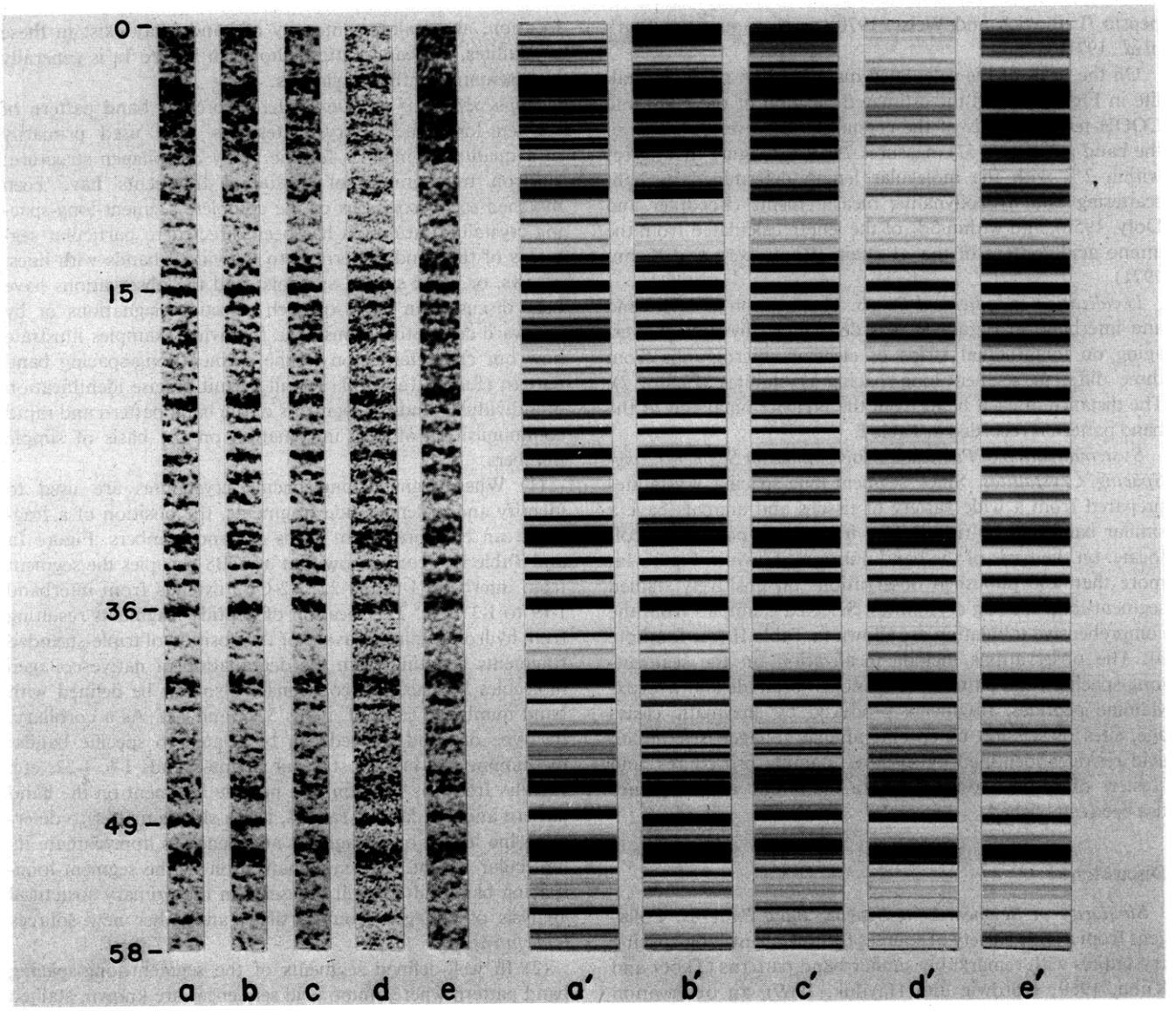


FIGURE 2: Electron micrographs of segment-long-spacing crystallites of calf skin collagen (a–e) and their corresponding optically averaged images (a′–e′). Micrographs a, b, and c are from different areas of a single crystallite; micrographs d and e are from two other crystallites. The five specimens taken together represent the averaged image of a crystallite *ca.* 500 Å in width. The five micrographs were brought to the same magnification by alignment of bands 15 and 49. The distance from the center of each band to the amino-terminal end (0) of the band pattern (Table I) was determined from measurements, with a steel machinists rule, on the optically averaged images a′–e′. Specimens were stained as for Figure 1; magnification, 509,000×.

residues to the distribution of stained and unstained bands. When the entire sequence of the collagen molecule is known, each residue, in both the α1 and α2 chains, probably can be assigned to a specific band.

(3) The well-characterized segment-long-spacing band pattern, used in combination with biochemical data, will facilitate investigations on enzymatic degradation of the collagen molecule. For instance, fragments of crystallites from native molecules cleaved by tadpole collagenase show that the scission occurs at interband I-41 (Table II, items 7 and 8). From the data in Figure 1a and Table I, we can calculate that the scission occurs in the α1 chain at a point about 658 Å from interband I-30, the location of the amino-terminal end of α1-CB7; and in the α2 chain at a point about 218 Å from band I-37, the amino-terminal end of α2-CB5. Using 2.91 Å as the average length of a peptide bond in the native conformation, we have estimated that the scission occurs in the vicinity of amino acid residue number 226 of the α1-CB7 peptide and near residue number 75 of the α2-CB5 peptide. According to available chemical evidence on residues at the site of cleavage, the scission should occur between glycine-leucine or glycine-isoleucine residues (Nagai *et al.*, 1964; Gross and Nagai, 1965). In the α1-CB7 peptide, a glycine-isoleucine sequence occurs at positions 221 and 222 (Fietzek and Kuhn, 1972[1]).

(4) The band pattern in Figure 1a can serve as a reference standard for analyzing the segment-long-spacing band patterns of uncommon collagens and for communicating the findings in unambiguous terms. The different staining intensities of certain bands in cartilage segment-long-spacing

[1] Fietzek, P. P., and Kuhn K. (1972), Max-Planck-Institut, Munich, personal communication.

TABLE I: Estimated Location of Bands in Segment-Long-Spacing Crystallites of Calf Skin Collagen.

Band No.[a]	Distance from NH_2-Terminal End ± Std Dev[b] (Å)	Band No.[a]	Distance from NH_2-Terminal End ± Std Dev[b] (Å)	Band No.[a]	Distance from NH_2-Terminal End ± Std Dev[b] (Å)	Band No.[a]	Distance from NH_2-Terminal End ± Std Dev[b] (Å)
0		15	868 ± –	I-29	1529	44	2338 ± 8
1	153 ± 8	I-15	896	30	1542 ± 5	I-44	2352
I-1	166	16	925 ± 4	I-30	1577	45	2367 ± 10
2	180 ± 6	I-16	955	31	1613 ± 5	I-45	2397
I-2	195	17	986 ± 8	I-31	1630	46	2428 ± 8
3	211 ± 8	I-17	1006	32	1647 ± 3	I-46	2442
I-3	225	18	1026 ± 2	I-32	1666	47	2457 ± 6
4	240 ± 10	I-18	1045	33	1685 ± 8	I-47	2467
I-4	277	19	1064 ± 5	I-33	1717	48	2478 ± 8
5	314 ± 8	I-19	1081	34	1750 ± 3	I-48	2523
I-5	337	20	1098 ± 7	I-34	1775	49	2568 ± 9
6	360 ± 10	I-20	1117	35	1800 ± 4	I-49	2614
I-6	383	21	1136 ± 4	I-35	1842	50	2660 ± 8
7	406 ± 12	I-21	1151	36	1884 ± 4	I-50	2671
I-7	426	22	1167 ± 8	I-36	1937	51	2683 ± 6
8	446 ± 5	I-22	1194	37	1991 ± 5	I-51	2693
I-8	490	23	1221 ± 10	I-37	2017	52	2704 ± 8
9	534 ± 5	I-23	1251	38	2043 ± 4	I-52	2724
I-9	553	24	1282 ± 8	I-38	2079	53	2744 ± 7
10	572 ± 5	I-24	1305	39	2116 ± 5	I-53	2765
I-10	613	25	1328 ± 6	I-39	2129	54	2786 ± 9
11	654 ± 3	I-25	1364	40	2143 ± 7	I-54	2809
I-11	687	26	1401 ± 6	I-40	2172	55	2813 ± 9
12	721 ± 2	I-26	1430	41	2202 ± 5	I-55	2842
I-12	746	27	1460 ± 4	I-41	2235	56	2872 ± 8
13	771 ± 4	I-27	1469	42	2269 ± 9	I-56	2909
I-13	788	28	1479 ± 3	I-42	2282	57	2947 ± 2
14	805 ± 2	I-28	1498	43	2296 ± 8	I-57	2965
I-14	836	29	1517 ± 11	I-43	2317	58	2983 ± 3

[a] Band numbers from Figure 1a. Interband numbers are preceded by "I." [b] Distance from the amino-terminal end (0) of the segment-long-spacing band pattern to the center of individual bands. The value for each dark band is the mean, ± the sample standard deviation, of measurements made on the five optically averaged images shown in Figure 2. All measurements were made from band 15. The center of individual interbands is considered to lie midway between adjacent dark bands.

reported by Trelstad *et al.* (1970) and confirmed by Stark *et al.* (1972) now can be described in terms of band numbers: in calf skin crystallites, dark bands 16–18 are noticeably more dense than dark bands 20–22, whereas in cartilage crystallites dark bands 16–18 are similar in density to dark bands 20–22; in calf skin crystallites band 24 is less dense than band 23, while in cartilage crystallites band 24 is as dense as band 23. Band 19 is usually faint and difficult to compare.

In preliminary observations on crystallites of phosphate-soluble collagen from guinea pig skin, we have found a greater than usual separation of bands 9 and 10.[2] By carefully screening segment-long-spacing crystallites of other unusual collagens it is likely that new variations in the segment-long-spacing band pattern will be discovered. Such variations may be expected to reveal sites where significant differences occur in the concentration of available charged groups.

[2] If new dark bands are identified, they can be designated with the number of the preceding dark band and a lower case letter, *e.g.*, band 16a.

(5) The information provided in Figure 1 and Table I also may be useful for evaluating models of molecular packing within native and polymorphic fibrils. From a correct model of molecular packing and our data on the segment-long-spacing band pattern, it should be possible to generate a fibrillar band pattern (by optical averaging or by computer methods) that is similar to the band pattern actually observed on stained fibrils. Such procedures may be important for understanding the molecular packing arrangement within collagen fibrils because eventually chemical data on the collagen molecule must be related to pictorial data on the collagen fibril. At present the segment-long-spacing crystallite is the only unit in the structural hierarchy of collagen that precisely links chemical and electron microscopic information.

Finally, our characterization of the segment-long-spacing band pattern applies only to positively stained crystallites. It does not correspond exactly to the bands of crystallites negatively stained at neutral pH where the band pattern apparently reflects topographical characteristics of the crystallite more than it does the distribution of reactive groups (Cox *et al.*, 1972).

TABLE II:[a] Selected Published Observations on Segment-Long-Spacing Crystallites Correlated with the Band Numbers Shown in Figure 1.

Source of Collagen	Location on Segment-Long-Spacing; Band No.[b]	Designation of Collagen Fragments	Comments and References
		I. Cyanogen Bromide Peptides	
1. Calf skin, α1	(I-6)–(I-22)	α1-CB8	Rauterberg and Kuhn (1968); Rauterberg *et al.* (1970)
2. Calf skin, α1	(I-22)–(I-30)	α1-CB3	Rauterberg and Kuhn (1968); Rauterberg *et al.* (1970)
3. Calf skin, α1	(I-30)–(I-45)	α1-CB7	Rauterberg and Kuhn (1968); Rauterberg *et al.* (1970)
4. Calf skin, α1	(I-45)–(57–58)	α1-CB6	Rauterberg and Kuhn (1968); Rauterberg *et al.* (1970)
		II. Hydroxylamine Reaction Products	
5. Rat skin, α1	(I-6)–(I-11)	HA1 fragment	NH_2-terminal part of α1-CB8 cleaved with hydroxylamine; Balian *et al.* (1971)
6. Rat skin, α1	(I-11)–(I-22)	HA2 fragment	COOH-terminal part of α1-CB8 cleaved with hydroxylamine; Balian *et al.* (1972)
		III. Enzyme Reaction Products	
7. Guinea pig skin, native	0–(I-41)	TC^A segment	Tropocollagen cleaved by tadpole collagenase; Harper *et al.* (1971)
8. Guinea pig skin, native	(42–43)–(57–58)	TC^B segment	Tropocollagen cleaved by tadpole collagenase; Harper *et al.* (1971)
9. Guinea pig skin, native	0–36	$TC_{67}{}^A$ segment	Tropocollagen cleaved by collagenase from rat uterus; Jeffrey and Gross (1970)
10. Guinea pig skin, native	0–34	$TC_{62}{}^A$ segment	Tropocollagen cleaved by collagenase from rat uterus; Jeffrey and Gross (1970)
11. Calf skin, native	36–52	780-A fragment	A native fragment isolated from tropocollagen partially digested by crude bacterial collagenase; Rauterberg *et al.* (1970)
12. Calf skin, α1	(I-45)–52	α1*(780)-CBC	A fragment of a segment-long-spacing crystallite prepared from the COOH-terminal piece of the α1*780 peptide (*cf.* item 11) cleaved with CNBr; Mark *et al.* (1970b)
		IV. Correlation of Amino Acid Sequence with Segment-Long-Spacing Bands	
13. Calf skin, α1	49	AA[c] residue No. 62–68	Specific stained bands of the segment-long-spacing fragment α1*(780)-CBC (see item 12) correlated with clusters of charged residues in the amino acid sequence of peptide fragment α1*(780)-CBC; Mark *et al.* (1970b)
14. Calf skin, α1	50	AA[c] residue No. 84	
15. Rat skin, α1	7	AA[c] residue No. 8–11	Specific stained bands of the segment-long-spacing fragment α1-CB8 (see items 1, 5, 6) correlated with clusters of charged residues in the amino acid sequence of fragments α1-CB8-HA1 and α1-CB8-HA2; Balian *et al.* (1971, 1972)
16. Rat skin, α1	8	AA[c] residue No. 21–24	
17. Rat skin, α1	15	AA[c] residue No. 65–74	
18. Rat skin, α1	16	AA[c] residue No. 86–93	

[a] A comprehensive form of this table, listing more than 100 published observations on segment-long-spacing crystallites, will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N. W., Washington, D. C. 20036. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche, referring to code number BIO-73-808. [b] The accuracy of these band designations is limited by the clarity of the published electron micrographs. Where two or more bands appear as a single line, the bands are designated by the first and last numbers of the included bands, *e.g.*, band 40–41. Interbands are designated by "I" and the number of the preceding dark band, *e.g.*, I-30. [c] Amino acid.

Acknowledgments

Mr. Robert Lindberg and Mr. Neil Gross assisted with the photographic procedures. Dr. Peter Fietzek, Dr. Klaus Kuhn, Dr. Gary Bailian, and Dr. Paul Bornstein generously shared, prior to publication, their data on amino acid sequences. Dr. M. V. King provided samples of myosin, phase B.

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