SUPRAMOLECULAR STRUCTURE OF POLYMORPHIC COLLAGEN FIBRILS

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ABSTRACT

Reconstituted cartilage collagen fibrils with an oblique banding pattern or with two types of symmetrical patterns, and reconstituted rattail tendon fibrils with a third type of symmetrical pattern were examined by electron microscopy and found to consist of narrow subfibrils having native-type cross-striations. Analysis of the four types of patterns by a graphic method of specific band matching revealed the orientation and axial relation of individual subfibrils and their component molecules. In fibrils with an oblique pattern, subfibrils have the same orientation and a regular 100-Å axial displacement. Observations on staining characteristics, folded fibrils, and transverse sections of embedded fibrils suggest that the obliquely banded fibrils are ribbonlike or layered structures. In the three types of fibrils with a symmetrical pattern, adjacent subfibrils are oppositely oriented and aligned within a 119-Å segment of the 670-Å major period. Considered together, the observations suggest that interaction sites on the surface of subfibrils (and perhaps on the surface of native collagen fibrils) occur in various patterns that are manifested according to the nature of the environment during fibril formation, and that such patterns can be mapped on the surface of subfibrils by noting the arrangement of subfibrils in polymorphic forms.

Collagen fibril formation in vivo is a complex process involving controlled synthesis, secretion, and assembly of collagen molecules as well as postsynthetic modification of the molecules both before and after the fibrils are formed. Our understanding of the molecular assembly aspects of the process, and of the structure of the resultant fibrils, has been significantly advanced by electron microscope studies on the “self assembly” of collagen molecules in vitro (23, 25, 26, 41), by physicochemical studies on the structure of the molecules (5, 39, 40, 49), and by amino acid sequence analysis of the component α chains (2, 3, 9, 17–20, 24, 29, 48). Such studies have revealed the axial relationship of molecules within the fibril (modified quarter-stagger arrangement); the triple-helical configuration of the molecule; and the complete primary structure of the α1 (type I) chain, approximately 60% of the α2 chain, and about 15% of the cartilage α1 (type II) chain. Correlation of electron microscope and amino acid sequence data in turn has led to new speculations on interactions between specific amino acid residues within the fibril (10, 12, 28, 50). The threedimensional organization of molecules within the fibril is still unclear, although it is thought to involve a tetragonal packing of four- to eight-stranded microfibrils (22, 27, 34, 35, 42, 49).

Recently, attention has been redirected to the study of polymorphic collagen fibrils for the new
information that they may provide on interaction characteristics of component molecules and on the three-dimensional structure of the native fibril. The study of such fibrils has been greatly simplified by the discovery that several polymorphic forms consist of narrow, native-type subfibrils packed in various arrangements (8, 15). Doyle et al. (13) already have begun a detailed analysis of polymorphic collagen fibrils on the basis of available primary structure data.

In the study described here, a previously proposed plan is carried out for analyzing the molecular arrangement in polymorphic collagen fibrils on the basis of specific band matching (6). This simple method of analysis has the advantage of utilizing information contained in all three α chains of the constituent molecules, and it requires no specific knowledge of amino acid sequences. The results establish, within ~25 Å, the axial arrangement of molecules in four types of polymorphic fibrils and suggest certain surface characteristics of the collagen fibril which may be important for understanding the packing of microfibrils and the interaction of fibrils with each other and with noncollagenous molecules.

**MATERIALS AND METHODS**

**Soluble Collagens**

Neutral soluble cartilage collagen (type II) from sternal cartilages of 2.5-wk old lathyritic chicks was purified and lyophilized as previously described (8, 44, 45). The collagen was homogeneous by the criteria of amino acid analysis of the native collagen molecules and of the isolated α chains, chromatographic behavior of the denatured collagen on carboxymethyl-cellulose and diethylaminoethyl-cellulose columns, and migratory characteristics of the α chains on polyacrylamide gel disc electrophoresis (8, 44, 45). Lyophilized collagen was dissolved in 0.5 M acetic acid (4°C) at a concentration of 0.1%.

Rattail tendon collagen was obtained by dissolving fresh tail tendons in 0.5 M acetic acid at 4°C. Insoluble material was removed by centrifugation, and the collagen concentration was adjusted to 0.1% by the addition of 0.5 M acetic acid. All collagen solutions were centrifuged at 105,000 g for 60 min at 4°C immediately before use.

**Reconstituted Fibrils**

Reconstituted cartilage collagen fibrils were prepared by dialyzing 1 or 2 ml of the collagen solution against 4 liters of 0.15 N sodium chloride at 4°C with continuous stirring for 8 h and against another 4 liters of the same solution for 12 h. Three drops of 1 N sodium hydroxide were added to the cold dialysate, and dialysis was continued at 23°C. Within 12 h a small amount of flocculent precipitate appeared and the pH of the dialysate was 5.4. During the next 2–3 days several more drops of sodium hydroxide were added to bring the pH of the dialysate to 6.5. Reconstituted rattail tendon fibrils were prepared by dialyzing 1–2 ml of the collagen solution against 1 liter of 0.25 N sodium chloride at 4°C for 8 h.

**Electron Microscopy**

In the usual procedure, drops of a fibril suspension were applied to 400-mesh carbon-coated grids held in locking tweezers and blotted dry with filter paper after 5 min. The fibrils were rinsed with several drops of water (or with several drops of the first staining solution), stained for 5 min with 0.1% uranyl acetate in 0.15 N sodium chloride, rinsed with water, and dried. The positively stained fibrils were then negatively stained with a drop of 4% aqueous sodium silicotungstate. This method of double staining will be referred to as "positive-negative" staining. In other cases, fibrils were: (a) stained only positively with the uranyl acetate solution or only negatively with 4% sodium silicotungstate, (b) fixed for 30 min with 0.5% glutaraldehyde in 0.15 N sodium chloride (pH 6) before staining, or (c) stained overnight with 0.2% aqueous uranyl acetate or 0.2% aqueous phosphotungstic acid. Unstained fibrils dried on carbon-filmed grids were shadowed at an angle of about 15° with gold-palladium.

To examine fibrils in thin sections, small pellets of fibrils, prepared by centrifuging the fibril suspension at 620 g for 2 min in an International Clinical Centrifuge (model CL) (International Scientific Instruments, Inc., Mountain View, Calif.), were fixed with 0.5% glutaraldehyde in 0.15 N sodium chloride (pH 6), postfixed with 0.5% osmium tetroxide, stained with 0.5% uranyl acetate (pH 4.7), dehydrated with ethanol, and embedded in Epon (16). Thin sections of the fibrils were examined unstained or after staining with uranyl acetate and lead citrate (46). Native fibrils in small blocks of cartilage matrix from sternal cartilages of lathyritic chicks were fixed, stained, and embedded as just described, or homogenized in 0.15 N sodium chloride and examined as positively or negatively stained whole mount preparations.

All specimens were examined with a JEOL JEM-100B electron microscope operated at 80 kV with a 20- or 40-μm objective aperture and with a decontamination device cooled with liquid nitrogen. Images were recorded on Kodak contrast lantern slide plates (Eastman Kodak Co., Rochester, N.Y.) at magnifications up to 100,000.

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1 Polymerization of Epon in the pellet of fibrils, and in tissue blocks, is greatly improved by substituting benzene for propylene oxide in the embedding procedure.
RESULTS

Reconstituted Cartilage Fibrils

Cartilage collagen, when precipitated from an acidic solution by slowly increasing the pH, forms mainly wide, tapered fibrils (tactoids) having an oblique banding pattern and narrow fibrils with indistinct bands. In addition, the precipitates usually contained: (a) tactoids consisting of two obliquely banded fibrils fused along one edge to form a "V" (chevron) arrangement, (b) fibrils with a symmetrical or "checkerboard" band pattern, and (c) complex fibrils in which oblique and symmetrical forms are fused. None of these forms were found in homogenates or thin sections of native cartilage matrix, the source of the soluble collagen.

Fibrils with an Oblique Banding Pattern: Every specimen grid examined contained tactoids with oblique bands inclined either to the right or to the left. The tactoids occurred in various sizes and attained a maximum length of about 6 μm. They measured up to 0.3 μm at the widest part and tapered to narrow ends of uniform diameter which extended for indeterminate distances. The tactoids were relatively straight and presumably rigid, whereas the narrow extensions appeared to be quite flexible. The oblique striations were inclined at 61° ± 5° (N = 302; range, 44–78) to the fibril axis (Fig. 1) and were seen in both glutaraldehyde-fixed and unfixed fibrils unstained or stained positively or negatively, in shadowed specimens (Fig. 6), and in thin sections of fixed and embedded fibrils (Fig. 7).

High magnifications revealed that the tactoids consisted of long, narrow, transversely banded subfibrils packed in parallel array and that the oblique banding pattern arose from a regular longitudinal displacement of adjacent subfibrils. The longitudinal axes of subfibrils lay approximately parallel to the fibril axis, but in many cases their lateral borders were not clearly defined, and their relation to the fibril axis could not be established. The subfibrils appeared to be structural entities and not just a surface lattice, since negative stain frequently collected between them (Fig. 1) and occasional fibrils with bent and frayed ends revealed the existence of longitudinal elements. The mean width of subfibrils is 138 Å ± 15 Å (N = 174; range, 108–183) from measurements made near the center of wide, well-preserved fibrils where the lateral boundaries are distinct (8).

Although subfibrils usually showed only the most conspicuous transverse bands, occasional well-preserved areas revealed clearly that subfibrils and native collagen fibrils have an identical intraperiodic structure. The example in Fig. 2 illustrates, in one major period of a single subfibril, 11 positively stained bands that match exactly to the intraperiod striations of a native fibril. The missing number V band is very faint and seldom visible even in native fibrils (7). Furthermore, the example shows the characteristic pattern of negatively stained fibrils superimposed on the positive staining pattern in the expected location (7). Hence, both subfibrils and native collagen fibrils have identical positive and negative staining characteristics, and presumably an identical organization of constituent molecules.

The axial polarity of individual subfibrils can be recognized directly in electron micrographs where the positively stained banding pattern is distinct and shows sufficient bands (Fig. 2 a). Where the pattern is less distinct or incomplete, the polarity can be established by noting certain staining characteristics: in fibrils stained both positively and negatively, the change in contrast between the "hole" and "overlap" zones is more abrupt at fibril band I than it is at band VI (cf. 7 and 10), and the prominent band IV lies closer to the edge of the hole zone at band VI than it does to that at band I. Such criteria indicate that parallel subfibrils in obliquely banded cartilage fibrils, and in the chevron patterns, have the same sense (Fig. 3).

For further analysis of the oblique banding pattern, each subfibril is represented by a diagram (Fig. 2 c) showing the mean location of 12 intraperiod bands in a "standard" native rattail tendon fibril (7). Transverse bands in the diagram are drawn either as narrow lines of uniform width to emphasize the mean location of each band (Figs. 2 c and 3 b) or in various widths to emphasize the relative width of different bands (Figs. 9 b and d; 11 a). This simple graphic method of representing subfibrils refines and clarifies the complex banding pattern by eliminating background noise and by supplying minor bits of information missing from individual subfibrils in the pattern, e.g., the location of faint bands II, V, and XII. Thus the method reveals details of band matching between subfibrils that may not be directly apparent in single electron micrographs or in optical diffraction diagrams of the patterns (14).

Favorable regions in electron micrographs of obliquely banded fibrils show that band no. I in one subfibril aligns with band no. XI in an adjacent...
FIGURE 1  Electron micrograph of an obliquely banded, reconstituted cartilage collagen fibril. The fibril consists of about 15 narrow, longitudinal subunits (subfibrils) each having alternating dark and light segments and a repeating period of 670 Å. A regular axial displacement of adjacent subfibrils accounts for the oblique pattern. Note the accumulation of negative stain between subfibrils (arrows). Whole mount preparation stained positively with 1% uranyl acetate and then negatively with 4% sodium silicotungstate. × 358,000.
FIGURE 2  (a) One major period of a single subfibril showing eleven positively stained transverse bands that match exactly the location of positively stained bands in one major period of a native rattail tendon fibril (b). Band V is not visible. "Hole" (dark) and "overlap" (light) zones occur at the expected location in the positively stained pattern (7). Arrows marked -NH₂ and -COOH indicate, respectively, the orientation of amino- and carboxyl-terminal ends of molecules that constitute the subfibril. Preparation as for Fig. 1. x 600,000. (b) Native rattail tendon fibril stained positively with 0.1% phosphotungstic acid followed by 0.5% uranyl acetate, x 600,000. (c) Diagrammatic representation of a native rattail tendon fibril banding pattern showing the mean location of 12 positively stained bands in a period of 662 Å and the location of the hole (dark) and overlap (light) zones (7). A detailed analysis of an oblique banding pattern (Fig. 1) can be effected by representing each subfibril (a) by a "standard" diagram (c) of the native fibril banding pattern because subfibrils have the native fibril-banding pattern. x 600,000.

one, an axial displacement of 101 Å (7), and that adjacent subfibrils have the same sense (Fig. 3 a). From this information, standard diagrams of two subfibrils can be arranged in exactly the same axial relation as adjacent subfibrils in the electron micrograph (Fig. 3 b); consequently, the axial relation of all bands in the two diagrams is established. With additional subfibril diagrams
aligned in the same manner, an extended two-dimensional diagrammatic representation of the oblique banding pattern is formed. Such diagrams show that the axial shift of 100 Å aligns primarily charged regions (dark bands) on one subfibril with noncharged regions (light bands) on an adjacent subfibril. Alignment of dark bands occurs only at I and X, VI and IV, and X and VIII (Fig. 3 b).

After positive staining with aqueous phosphotungstic acid or uranyl acetate for periods of up to 12 h, the obliquely banded fibrils show a complex pattern of broad, oblique, dark and light bands, and conspicuous narrow transverse bands (Fig. 4). According to Fig. 3 b, the oblique bands correspond to the hole and overlap zones, and appear in reverse contrast, presumably because the overlap contains one-fifth more protein than the hole and has more charged groups available for binding heavy metal stains. The conspicuous narrow dark bands, located in the hole regions, result from the transverse alignment of bands I, X, and perhaps IX and X in adjacent subfibrils. They are not in transverse alignment with similar bands in adjacent hole zones, but are misaligned by 30–40 Å.
The misalignment results from the fact that the major repeating period of individual subfibrils (670 Å) is not a multiple of the 101 Å axial displacement that occurs between adjacent subfibrils. Frequently, the misalignment is overlooked, especially at low magnification where the fibrils appear to have perfect transverse banding with a period of 100 Å (cf. Doyle et al. [13]).

In six instances where obliquely banded fibrils were clearly folded over, the oblique pattern showed a reversal of "hand" (Fig. 5).

Thin sections of the fixed and embedded fibrils, stained with uranyl acetate before embedding and with uranyl acetate and lead citrate after thin sectioning, show right- and left-handed oblique (Fig. 7) or chevron patterns that apparently are identical with the patterns observed in positively stained whole-mount specimens (Fig. 4). Transverse sections of fibrils in the same embedded samples show a wide variety of profiles which range in overall diameter from 150 to 3,000 Å (Figs. 7 and 8). The largest sizes are highly irregular and frequently contain internal "cavities" which suggest that the fibrils may be complex tubular structures (Figs. 8 a and f). Intermediate sizes tend to be more regular (Fig. 7). In the larger fibrils, it is relatively common for both the external and internal profiles to have a variety of angular segments (Figs. 8 a, b). The smallest profiles are approximately circular and usually appear to be joined side by side in irregular curved or branching configurations (Figs. 8 c-e). The internal structure of the fibrils is uniformly granular in the specimens examined, and it shows no obvious evidence of subfibril organization.

FIBRILS WITH A SYMMETRICAL BANDING PATTERN: With positive staining, occasional cartilage fibrils show a characteristic symmetrical banding pattern (Fig. 9 a) also observed by others (13, 15, 38). The repeating period of 670 Å contains a conspicuous doublet band, a less conspicuous intermediate doublet bands in the pattern arise from the major bands III, IV, VI, and VIII; the less conspicuous intermediate doublet is derived from two XI bands, with a slight contribution from number XII; and the four faint bands originate from II, IX, and X. The segments of increased width and density in the faint bands correspond to the major no. 1 bands. Hence, in general, conspicuous doublet bands result from alignment of equally dense bands in oppositely oriented subfibrils, and the bands that vary in density result from alignment of bands of unequal density.

With positive-negative staining, the symmetrical fibrils have a checkerboard appearance which clearly shows oppositely oriented subfibrils. The thickness of such fibrils cannot be established from the two-dimensional projections studied, but the appearance of the staining pattern suggests that the fibrils are relatively thin. Transverse sections of the fibrils have not been studied because they cannot be distinguished from those of obliquely banded fibrils.

Another type of symmetrically banded fibril, designated DPS-II, occurs rarely among the obliquely banded fibrils (Fig. 10). It consists of oppositely oriented subfibrils, as in the DPS-I pattern, but adjacent subfibrils are aligned at bands IV and IX instead of at bands XI and XII. This axial alignment matches primarily major charged regions (heavy dark bands) with minor dark bands or with interbands. With positive-negative staining, the band pattern has a checkerboard appearance that is similar, but not identical to that of the DPS-I fibril.

Reconstituted Rattail Tendon Fibrils

When rattail tendon collagen in 0.5 M acetic acid is dialyzed against a 0.25 N sodium chloride solution, practically all of the fibrils that form have a symmetrical banding pattern (DPS-III) that is
distinctly different from that of reconstituted cartilage collagen fibrils. With positive staining, the DPS-III pattern consists of a wide conspicuous doublet band, which repeats every 670 Å, and a pair of narrower intermediate doublet bands (Fig. 11b). This symmetrical pattern can be generated from two standard diagrams of the native-type pattern oppositely oriented and aligned at bands V and VI (Fig. 11a and b). The wide doublet is formed by bands I and XI, and the pair of narrower doublets by bands III, IV, VIII, and IX. The remaining bands are usually blurred and do not contribute significantly to the pattern. With positive-negative staining, a wide, dense, transverse band occurs regularly at 670 Å intervals along the fibril (Fig. 11c). Graphic analysis of the pattern indicates that this band corresponds to a 100 Å overlap of hole zones between bands I and XI in oppositely oriented subfibrils (Fig. 11a). Constituent subfibrils must be very narrow, for checkerboard patterns have not been observed in such fibrils. Certain specimens in which bands V-VII are visible, however, provide additional evidence that the symmetrical pattern arises from oppositely oriented subfibrils aligned at bands V and VI (Fig. 11d).

**Molecular Arrangement in Polymorphic Fibrils**

A first approximation to the axial alignment of molecules within the four types of polymorphic fibrils can be made from observations on subfibril band matching and from the known relation between the positively stained banding pattern of native fibrils and the modified quarter-stagger arrangement of molecules (7, 10, 11, 25, 28). The subfibril banding pattern and a corresponding modified quarter-stagger array of its component molecules may be represented diagrammatically as a coordinated unit, e.g., Fig. 12, and two such parallel units can be arranged in various ways to reproduce subfibril band matching in the four polymorphs. The oblique band pattern is represented by two units of the same polarity aligned at subfibril bands I and XI (Fig. 12), and the DPS-I, DPS-II, and DPS-III patterns are represented by oppositely oriented units aligned at bands XI and XII, IV and IX, and V and VI, respectively. Alignment of two subfibril bands in adjacent units automatically establishes the axial relation between the two quarter-stagger representations of constituent molecules. For example, in the DPS-III pattern (bands V and VI aligned, Fig. 11a), the axial distance from band I-I (location of the amino-terminal end of the helical part of the molecule (10)) to band V in one subfibril is 225 Å, and the distance from the aligned VI band in the adjacent and oppositely oriented subfibril to band I-I is 266 Å (7); hence, amino-terminal ends of such molecules in oppositely oriented quarter-stagger arrays are overlapped by 491 Å. Through the intermediary of matched subfibril banding patterns, it is possible, therefore, to compare the axial distance between loci on any collagen molecules in a polymorphic fibril. Furthermore, since individual residues in the amino acid sequence of the α1 chain have been related to bands of the native fibril (2, 3, 6, 7, 10, 47), the axial relation between α1 residues of any molecules within a fibril can be estimated within ~25 Å. It should be noted that such calculations reveal only the axial distance between loci projected onto the axis of a polymorphic fibril; conclusions about relations between loci on spe-

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**FIGURE 4** Segment of an obliquely banded fibril stained positively with phosphotungstic acid and uranyl acetate. The wide, oblique dark and light bands correspond, respectively, to the overlap and hole zones in regularly displaced subfibrils. The narrow, dark, transverse lines (arrows) correspond to aligned bands I, XI, and perhaps IX and X, in the hole zones of adjacent subfibrils. × 506,000.

**FIGURE 5** A folded, reconstituted cartilage collagen fibril showing a change of hand in the oblique pattern. At 1 the oblique bands are inclined "downward" from the edge, and at 2 they are inclined "upward." Specimen prepared as for Fig. 1. × 143,000.

**FIGURE 6** Reconstituted cartilage collagen fibril, shadowed with gold-palladium, showing the "chevron" band pattern. Whole mount preparation, unstained. × 76,400.

**FIGURE 7** Thin section of reconstituted cartilage collagen fibrils showing the oblique banding pattern in a longitudinally sectioned fibril, and a great variation in the overall diameter and profile of transversely sectioned fibrils (arrows). Pellet of fibrils fixed with glutaraldehyde-osmium tetroxide, stained with uranyl acetate at pH 4.7, and embedded in Epon. Thin section stained with uranyl acetate, lead citrate. × 82,000.
FIGURE 8 (a-f) Transverse sections of reconstituted cartilage collagen fibrils. The large irregular fibrils (a, b, f) have a uniform, granular texture and contain internal "cavities." Note the small surface irregularities (arrows in a) that could represent individual subfibrils, and square profiles (b) that may reflect the packing of subfibrils. Narrow, irregular curved (d, e) and branched (e) structures may represent transverse sections of individual subfibrils (arrows in e) joined side by side. Specimen prepared as for Fig. 7. × 141,000.

Specific collagen molecules require knowledge of the three-dimensional structure.

DISCUSSION

Obliquely Banded Fibrils

General Structure: Fibrils with right- or left-handed oblique banding evidently represent "front" and "back" views of a ribbonlike structure. A ribbonlike fibril, negatively stained, would be expected to show a change of hand when folded over (Fig. 5), whereas one with a helical surface structure would show a change of hand only if negative staining occurred on the fibril surface facing the supporting film in the segment on one side of the fold and on the surface facing away from the film in the segment on the other side, an unlikely occurrence. Furthermore, if obliquely banded fibrils had a helical surface structure, one would expect to see a crossed pattern in positively
FIGURE 9 (a) A complex, reconstituted cartilage collagen fibril showing both the symmetrical DPS-I and chevron banding patterns. The symmetrical part of the pattern has a 670 Å repeating period which consists of a conspicuous pair of doublet bands, a narrow, less conspicuous intermediate doublet, and four faint bands of uneven density. Positive staining with phosphotungstic acid and uranyl acetate. × 189,000. (c) An enlarged segment of the symmetrical pattern in (a) aligned with two oppositely oriented diagrams of the standard native fibril banding pattern (b and d). The diagrams show that the conspicuous pair of doublet bands are formed by fibril bands III, IV, VI, and VIII of two oppositely oriented subfibrils; the less conspicuous intermediate doublet is formed primarily by the XI bands; and patches of increased density (arrows) by the number I bands of subfibrils about 140 Å in width. The apparent absence of band VII is unexplained. N indicates the amino-terminal orientation of the fibril diagrams. × 500,000.
Stanley, whole-mount or longitudinally sectioned fibrils, yet such patterns were not found in this study (cf. Figs. 4 and 7). Doyle et al. (13, Plate VIII) show a fibril that has a faint crossed pattern, but such patterns could represent fortuitously superimposed or longitudinally folded fibrils, and apparently they do not occur as frequently as would be expected with helical fibrils. It is unlikely that the right- and left-handed forms represent distortion artifacts created during preparation of the specimens, for the same oblique pattern occurs in fibrils fixed with glutaraldehyde, stained before dehydration, and examined as whole mounts or thin sections. Finally, the axial displacement of subfibrils is extremely uniform in fibrils of different widths (8, 14), and tactoids of skin collagen applied to grids and stained in the same manner used in this investigation (4, 31, 32) show transverse striations without evidence of systematic axial distortion.

Similar, and perhaps related obliquely banded fibrils have been observed by Kühn et al. (30) in preparations of reconstituted calfskin collagen fibrils. Their published example shows a complex fibril consisting of a central longitudinal segment having a 120 Å transverse periodicity, and of lateral oblique segments in which a subfibril structure is not visible. It would be of interest to repeat this observation to determine whether type I collagen is indeed capable of forming the same oblique pattern observed with cartilage collagen. Doyle et al. (13) speculate that the 120-Å periodic structure is a special case of the obliquely banded fibril (D/6), but our preliminary observations on fibrils formed by dialyzing an acetic acid solution of rattle tendon collagen against a 4% sodium chloride solution suggest that it also could arise from DPS-III fibrils axially displaced by D/3.

The exact experimental conditions required for the formation of obliquely banded fibrils are unknown. Since the fibrils form as the pH and temperature of the collagen solution are slowly increasing, it is possible that at certain conditions of pH, ionic strength, and temperature, the molecules aggregate in the modified quarter-stagger arrangement to form subfibrils, and that at other conditions (higher pH) the surface charge or conformation of the subfibrils is changed and they aggregate in a staggered arrangement. Other mechanisms, however, also must be considered (vide infra).

SUBFIBRIL ORGANIZATION: Although the three-dimensional structure of obliquely banded fibrils is not yet established, certain information can be obtained from staining characteristics and from transverse sections of embedded fibrils. If we assume a simplifying model in which each subfibril has four interacting surfaces, a, a', b, b' (Fig. 13), the staggered arrangement of subfibrils indicates that two opposing surfaces of a subfibril (e.g., a, a') have transverse asymmetry with respect to their interaction with adjacent subfibrils; and the native banding pattern of subfibrils, which is clearly demonstrated after positive or negative staining, suggests that the two remaining surfaces (b, b') either interact with adjacent subfibrils in transverse register or fail to interact with other subfibrils. Thus, the surface of a subfibril is not uniform with respect to its ability to interact with other subfibrils.

Fibrils with a chevron pattern (Figs. 6 and 9 a) are not inconsistent with these models. Although

FIGURE 10 Reconstituted cartilage collagen fibril showing the DPS-II "checkerboard" band pattern which arises from oppositely oriented subfibrils. The three dots, from above, mark aligned bands IV, IX, and IV in three subfibrils. The amino-terminal direction of the middle subfibril is to the left. Preparation as for Fig. 1. Line represents 500 Å × 384,000.
Symmetrical band patterns (DPS-III) of reconstituted rattail tendon collagen fibrils. (a) Diagrams of two standard fibril banding patterns oppositely oriented and aligned at bands V and VI. (b) Fibril positively stained with uranyl acetate. Bands I and XI in oppositely oriented subfibrils form the wide doublet band repeating every 670 Å (dotted lines). The pair of intermediate doublets are formed by bands III, IV, VIII, IX, and perhaps VII. (c) Fibril after positive-negative staining with uranyl acetate and sodium silicotungstate. The wide, dense bands (dotted lines) correspond in location to the area between the doublet bands in (b) and represent overlapped hole zones in oppositely oriented subfibrils (a). (d) A complex fibril formed by dialyzing soluble rattail tendon collagen in 0.1 M citrate buffer, pH 3.7 against distilled water. A narrow, native-type fibril (longitudinal axis indicated by short arrows) appears to be superimposed on a wider native type fibril of opposite orientation. The asymmetric distribution of bands V, VI, and VII (long arrows) indicates an opposite orientation of the two fibrils and establishes their alignment at bands V and VI. Note the ordinary, native-type, polarized banding pattern at the lateral edges of the wide fibrils, and except for bands V–VII, the DPS-III pattern formed by major bands of the superimposed fibrils (between short arrows). Positive staining with phosphotungstic acid and uranyl acetate. (a–d) × 376,000.

FIGURE 11 Symmetrical band patterns (DPS-III) of reconstituted rattail tendon collagen fibrils. (a) Diagrams of two standard fibril banding patterns oppositely oriented and aligned at bands V and VI. (b) Fibril positively stained with uranyl acetate. Bands I and XI in oppositely oriented subfibrils form the wide doublet band repeating every 670 Å (dotted lines). The pair of intermediate doublets are formed by bands III, IV, VIII, IX, and perhaps VII. (c) Fibril after positive-negative staining with uranyl acetate and sodium silicotungstate. The wide, dense bands (dotted lines) correspond in location to the area between the doublet bands in (b) and represent overlapped hole zones in oppositely oriented subfibrils (a). (d) A complex fibril formed by dialyzing soluble rattail tendon collagen in 0.1 M citrate buffer, pH 3.7 against distilled water. A narrow, native-type fibril (longitudinal axis indicated by short arrows) appears to be superimposed on a wider native type fibril of opposite orientation. The asymmetric distribution of bands V, VI, and VII (long arrows) indicates an opposite orientation of the two fibrils and establishes their alignment at bands V and VI. Note the ordinary, native-type, polarized banding pattern at the lateral edges of the wide fibrils, and except for bands V–VII, the DPS-III pattern formed by major bands of the superimposed fibrils (between short arrows). Positive staining with phosphotungstic acid and uranyl acetate. (a–d) × 376,000.
Figure 12  Diagram showing the axial relation of collagen molecules in an obliquely banded fibril (Fig. 1). Diagrams of two subfibrils (1, 2) show the orientation and axial relation of adjacent subfibrils (cf., Fig. 3 b). Arrows represent the modified quarter-stagger arrangement of molecules within subfibrils 1 and 2 (7). Arrowheads identify the amino-terminal end of the molecules. Short transverse lines on the arrows (numbered 9-10, 23, 36, and 49; and aligned with subfibril band X1) mark the location of conspicuous SLS bands which correspond to specific loci on the collagen molecule (6, 10).
FIGURE 13 Diagram showing transverse sections of three hypothetical subfibrils. With a-a' surface interactions, adjacent subfibrils are displaced axially by 100 Å as in an obliquely banded fibril (Fig. 1), but with b-b' surface interactions they are aligned in transverse register. In some cases, the b-b' surfaces may not interact with other subfibrils (cf. Fig. 8 d and e).

we would expect to find one subfibril at the twin axis of a chevron pattern in which the a and a' surfaces bind adjacent subfibrils in a symmetric rather than in an asymmetric relation, careful examination of the chevron pattern usually reveals a slight overlapping of the right- and left-handed parts of the pattern. The overlapping suggests that some subfibrils at the center of the chevron are bonded in register on their b and b' surfaces.

The examination of transverse sections of fixed and embedded fibrils has not revealed the packing arrangement of subfibrils. The fibrils of large diameter (2,000-3,000 Å) have a regular, granular internal structure that gives no obvious indication of subfibril organization; however, small rounded irregularities at the fibril surface may represent individual subfibrils (Fig. 8 a), and angular segments of the profiles could reflect the packing of subfibrils. The smallest, dense fibrils (diam, 200 Å) are approximately circular in transverse section and apparently fuse with one another to form irregular narrow profiles (Fig. 8 c-e) that could be transverse sections of obliquely banded fibrils one subfibril in thickness. Such fibrils could account for the change of hand observed in fibrils that are folded over (Fig. 5).

ORGANIZATION OF "SUBFIBRILS" IN TISSUE: The observations on obliquely banded fibrils also lead to speculations on the structure of some abnormal collagen fibrils found in living tissues. In dermatosparaxis, a hereditary disease of cattle and sheep involving a defect in the conversion of procollagen to collagen (33), the dermis contains numerous abnormal collagen fibrils, which in transverse section have distorted "T" or "H" profiles and a normal range of overall width (300-1,000 Å) (21, 37). The "arms" or "branches" of the distorted profiles are 100-200 Å thick. In longitudinal view, the fibrils have a "loose" twisted appearance and native-type transverse striations. Such abnormal fibrils may represent narrow collagen fibrils (subfibrils) that interact primarily on one pair of opposing surfaces. In terms of the model shown in Fig. 13, the interactions would occur only on the b and b' surfaces, i.e., with fibril bands in transverse register, because the fibrils have transverse cross-striations rather than an oblique pattern. The normal range of overall fibril width suggests that the fibril "diameter regulating mechanism" functions normally in animals with dermatoosparaxis. Sheetlike aggregates found in certain preparations of reconstituted rat skin collagen (23) may be other examples of narrow fibrils that interact on only one pair of opposed surfaces.

Symmetrical Fibrils

Three distinct types of symmetrical fibril patterns can be generated by combining oppositely oriented native fibril band patterns (Figs. 9-11). The DPS-I and DPS-III patterns, generated entirely from quantitative electron microscope data on band locations in native fibrils, are similar to the two symmetrical models of Doyle et al. (13), which are based on synthesis of the fibril patterns from the distribution of charged residues in the a1 chain of rat/calfskin collagen. Their data show that the amino-terminal end of the helical part of the collagen molecules in the two patterns overlaps by 150 and 165 ± 5 residues, respectively. Since one residue projects 2.86 Å on the molecular axis (28), 150 residues represents an overlap of 429 Å, and 165 residues represents an overlap of 472 Å. By comparison, electron microscope data show that the amino-terminal end of the helical part of the collagen molecules in the two patterns overlaps by 150 and 165 ± 5 residues, respectively. Since one residue projects 2.86 Å on the molecular axis (28), 150 residues represents an overlap of 429 Å, and 165 residues represents an overlap of 472 Å. By comparison, electron microscope data show that the amino-terminal end of the helical part of the molecule, located at fibril band I-I (10), aligns near band X in the DPS-I pattern and corresponds to an amino-terminal overlap of 439 Å (154 residues). In the DPS-III pattern, bands I-I and I-X are aligned, and the overlap is 491 Å (172 residues). Since the electron microscope data include information on the charge distribution in all three a chains of the constituent molecules, and since the data of Doyle et al. (13) may require slight revision after the complete amino acid sequence of the a2 chains and the three-dimensional distribution of residues are established, the two methods of analysis in these cases give essentially the same results. In addition to descriptions of the DPS-I and DPS-III patterns, the findings also include a new symmetrical pattern (DPS-II)
in which bands IV and IX are aligned and the amino-terminal helical ends of equivalent molecules in adjacent subfibrils are separated by 558 Å (195 residues) (Fig. 10).

All three of the symmetrical patterns have a repeating period of 670 Å and mirror planes of symmetry near bands I-V and XII. In the DPS-III pattern, one plane of symmetry occurs exactly at band I-V. A subfibril translation of 53 Å (19 residues) from band I-V in the carboxyl-terminal direction forms and DPS-I pattern, as was noted by Doyle et al. (13), and a translation of 66 Å (23 residues) from band I-V in the amino-terminal direction forms the DPS-II pattern. Hence, the axial alignment of subfibrils in all three patterns is limited to a range of 119 Å (42 residues) within the period of 670 Å (234 residues).

It is interesting to note that the axial translation between subfibrils in the obliquely banded fibril, the difference in overlap between molecules in the DPS-I and DPS-II patterns, and the length of the nonhelical carboxyl-terminal extension of the cartilage collagen molecule (43) all are about 100 Å. It may be significant that all three symmetrical patterns have a mirror plane of symmetry near the edge of the hole zone at band VI, a site where the carboxyl-terminal cross-link, two disaccharides, the molecular site cleaved by tadpole collagenase, and a high concentration of large hydrophobic residues are clustered (7, 10).

The DPS-III banding pattern represents a common aggregation state of subfibrils, for it has appeared frequently in the literature (13). A comparison of photographic enlargements (magnification, 400,000) of symmetrical patterns published by Kühn et al. (31), Bard and Chapman (4), and Leibovitch and Weiss (32) shows that all three are identical to the symmetrical pattern shown in Fig. 11 b. Furthermore, fibrils that show a 220-Å symmetrical repeating period in shadowed preparations (23) also show the DPS-III pattern with positive staining. Doyle et al. (13) correctly predicted that such fibrils could be D-periodic, symmetrical structures, but they based their explanation of it on the DPS-I cartilage pattern. The symmetrical fibril patterns shown by Nemetschek (36) also have the DPS-III pattern, but in addition they have a dense, crooked band between the pair of faint doublet bands. The additional band apparently represents an accumulation of phosphotungstic acid stain at the slightly separated carboxyl-terminal edges (band VI) of the hole zones in adjacent and oppositely oriented subfibrils (cf. Fig. 11 a). When reconstituted rattail tendon fibrils with the DPS-III pattern are stained only with phosphotungstic acid and rinsed very briefly with water, a similar, but less dense band appears in the same location.

Numerous reports in the literature describe narrow collagen fibrils with a periodicity of about 100 Å or 200 Å in developing tissues (see summary of references in reference 1). The observations on polymorphic fibrils suggest that such periodicities may represent different interpretations of the native band pattern rather than different types of fibrils. In very narrow native collagen fibrils viewed at low resolution, bands III and IV, and XI and 1' frequently appear as conspicuous doublet bands while the other bands are faint or invisible. The doublets could easily be interpreted incorrectly as representing a regular period of about 100 Å (from band III to IV, and from XI to 1') or of about 200 Å (from doublet XI–I' to doublet III–1IV). The possibility also exists that some newly formed embryonic collagen fibrils consist of oppositely oriented microfibrils aligned in the DPS-III form (Fig. 11 a). The three doublet bands per major period could represent the 220-Å periodicity reported. Such fibrils could have special biological significance during development.

**Molecular Interactions in Polymorphic Fibrils**

Finally, I would like to propose for consideration that variation in the collagen environment during fibril formation affects the axial packing of microfibrils and consequently the packing of subfibrils.

According to the Smith model (42), collagen molecules are arranged in five-stranded microfibrils, and the microfibrils are aggregated laterally with major periods in register and with adjacent microfibrils "randomly" staggered by an "integer multiple" of the major period. In a preliminary study of "uniform" rather than "random" staggering, each of the five possible axial relations between microfibrils was examined in two-dimensional representations of 2- to 10-stranded microfibrils (cf. the two-dimensional representations of five-stranded microfibrils in Fig. 12). In each case the molecules were distributed in a specific pattern according to the type of axial relation between interacting molecules of adjacent microfibrils.

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4 In the amino- to carboxyl-terminal sense, bands I and I' are the first bands in consecutive major periods (Fig. 2 c).
How the five different types of microfibril interaction determine the distribution of molecules on the surface of a three-dimensional fibril is now being studied in models, for the pattern of molecular distribution on the surface of collagen fibrils may determine how fibrils (subfibrils) interact. Information on fibril surface characteristics also could be helpful for understanding the internal structure of native and polymorphic collagen fibrils and the relationship between the fibril surface and proteoglycans of the extracellular matrix.

ADDENDUM


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