Hagfish Slime Gland Thread Cells.
II. Isolation and Characterization of Intermediate Filament Components Associated with the Thread

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ABSTRACT The slime glands of hagfish have two major cell types, gland thread cells (GTCs) and gland mucous cells (GMCs), both of which upon contact with water contribute to the formation of an abundant quantity of viscous mucus. In previous studies we reported a method for the isolation of GTCs and showed that each ellipsoidal thread cell normally contains a single tapered thread which is uniquely coiled into a space-saving conformation and occupies most of the cell volume. Subsequently, the developing thread was found to consist mainly of intermediate filaments (IFs) aligned in parallel not only to one another but also to a far fewer number of interspersed microtubules (see accompanying paper). In the present report, urea extracts of GTCs were purified and characterized to establish the properties of thread components. One major (α) and two minor (β, γ) components prepared by anion exchange chromatography were shown to have similar apparent molecular weights of 63,500 ± 500 daltons but different isoelectric pH values (α, 7.56; β, 5.67; γ, 5.31). Although the amino acid content of α differed significantly from β and γ, each of the three was highest in Gly, relatively high in Glx, Ser, Thr, Asx, Ala, Val, and Leu, and relatively low in Cys/2 and Trp. The amino acid compositions of β and γ were very similar, and only β showed evidence of carbohydrate. The threonine content of the α component was higher than has been reported for IFs of different origin, and the high content of hydroxyamino acids (18, 19 residues per 100) in α, β, and γ has been approached only by several IF polypeptides from human or bovine epidermal keratins. Mixtures of the purified components formed 9–11-nm filaments in vitro. The results indicate that the hagfish thread cell is a rich source of IFs, which have a structure that facilitates formation of macrofibrils within the cell.

The hagfishes are very primitive vertebrates, as judged by numerous morphological and physiological criteria (1), and have a notable ability to secrete large quantities of mucus (2–7). The mucus-synthesizing capacity is attributable in part to three epidermal cell types which collectively approach 98% of total cells in middorsal-lateral regions of the integument (4). The mass of the mucus, however, and most of the mucous proteins are products of the numerous epidermally derived slime glands located along both ventro-lateral regions of the integument (1, 5, 6). Exclusive of the surrounding capsular material, each gland mainly contains gland mucous cells (GMCs) and gland thread cells (GTCs), both of which when expelled through the pore of the gland rupture upon contact with water and quickly interact to form viscous mucus (5, 6). We have previously described (5, 6) the potential usefulness of the hagfish as an experimental model for studying the biology of mucus and we have reported a procedure for isolation of intact GTCs and vesicles from GMCs. The mature

Abbreviations used in this paper: DTT, dithiothreitol; GMC, gland mucous cell; GTC, gland thread cell; IF, intermediate filament; PE, precursor extract.
ellipsoidal GTC is a unique, highly specialized cell in which 60–80% of the cell volume is occupied by a single tangled thread, which may approach 60 cm in length (5). Scanning electron microscopy of both intact and partially ruptured GTCs reveals that the thread is concisely compartmentalized by means of a repetitively looped, coiled conformation (5). The ultrastructure of a thread, at one stage of maturity, may show 20 or more microtubules in parallel alignment with numerous intermediate-sized filaments which make up the bulk of the thread material (8, 9). Very limited information exists on the molecular weight and amino acid composition of thread polypeptides. Analysis of partially purified “fiber” protein by Ferry (10) in 1941 showed values of -0.42% for cystine, <0.2% for tryptophan, and 5.5% for tyrosine. Hashimoto (11), as cited in reference 12, reported that the fibrous component had an M of 14,000–18,000 and was rich in leucine, aspartic acid, and glutamic acid. In contrast, we indicated previously (5) that the principal thread polypeptide had an M of ~63,500 and was rich in glycine, hydroxyamino acids, and glutamate (Glx), but low in half-cystine and tryptophan (7). In view of the unusual size and ultrastructure of the thread and its possible relationship, at least in the case of the hagfish, to the physical and biological properties of mucus, we report a procedure for the isolation and characterization of the major components found in urea extracts of hagfish GTCs. The major polypeptide has an M of ~63,500 and represents the principal component of IFs from thread.

MATERIALS AND METHODS

Materials: Items for gel filtration (Bio-Gel P-300, 50–100 mesh), anion exchange chromatography (DEAE-Bio-Gel A, 100–200 mesh) and deionization of urea (mixed bed resin AG 501-X8) were obtained from Bio-Rad Laboratories, Richmond, CA. Stock urea solutions prepared with glass-distilled water were filtered twice through AG 501-X8 resin, passed through a 0.2-μm Nalgene filter (Nalge Co., Rochester, NY), and stored at 4°C or frozen prior to use.

Purification of Hagfish Cland Thread Protein: Adult Pacific hagfish (Eptatretus stouti, 25–40 cm in length (Pacific Bio-Marine Laboratories Inc., Venice, CA), were kept in 30-gal refrigerated aquaria under conditions described earlier (3, 4). The fish were anesthetized and prepared for electrostimulation as before (3, 4, 6). The electrodes from a Grass SD 9 stimulator (Grass Instrument Co., Quincy, MA; frequency of 16 pulses per sec [pps], 10 ms delay, 1.4 ms duration, 20–25 V) were applied near each pore of the two linear rows of pores.叙事 was exercised to prevent possible contamination from gill slits by avoiding stimulation of adjacent pores. The white drops of exudate were collected for 15 min to yield a pellet containing mainly GTCs and some vesicles. The filtrate was centrifuged at 4°C for 15 min to yield a pellet containing mainly GTCs and some vesicles. The supernatant (SV), which contained the soluble components and mucous vesicles, was saved for SDS-PAGE. The pellet was resuspended in 1 M (NH4)2SO4 solution (pH 7.4) and kept at 4°C. Usually the exudates from four to eight hagfish were admixed. After careful stirring to disperse the mixture of unruptured GTCs and stabilized vesicles from GTCs, the suspension was filtered through cheesecloth to remove large aggregates of debris, including some ruptured GTCs and vesicles. The filtrate was centrifuged at 1,000 g for 15 min to yield a pellet containing mainly GTCs and some vesicles. The supernatant (SV), which contained the soluble components and mucous vesicles, was saved for SDS-PAGE. The pellet was resuspended in 1 M (NH4)2SO4 and centrifuged to obtain a pellet enriched in intact but membranecless GTCs. The pellet was resuspended and filtered through a nylon mesh screen (HC-3-41, openings of 41 μm; Testo Inc., Elmsford, NY), which retains GTCs but permits the passage of vesicles. The GTCs were backwashed into 30% sucrose/1 M (NH4)2SO4, which resulted in a suspension of intact GTCs, which was centrifuged into a pellet. The GTCs were solubilized at 4°C in 20 ml of urea-Tris (8 M urea, 20 mM Tris, 1 mM dithiothreitol [DTT], 1 mM EDTA, 0.5 mM phenylmethylsulfonylfluoride [PMSF], adjusted to pH 8.0) and centrifuged (500 g, 30 min) to remove undissolved material. The supernatant was either dialyzed (4–8 h, 4°C) against Dulbecco’s solution (1.47 mM KH2PO4, 8.06 mM Na2HPO4, 2.68 mM KCl, 136 mM NaCl, 0.5 mM PMSF, 10 mM DTT, 3.07 mM sodium azide, adjusted to pH 7.4) or added directly to Dulbecco’s to induce precipitation. The suspension was centrifuged, washed with Dulbecco’s, and resolubilized in urea-Tris. Three additional precipitation/washing/solubilization cycles yielded the precursor extract (PE), which contained ~10–20 mg protein/ml. The protein content in urea solutions was measured spectrophotometrically by reference to A450nm = 5.71, a relation-

ship derived by direct weighing of purified protein. Similar hagfish protein samples assayed by dye absorption (Bio-Rad Laboratories) yielded disparate values at A450nm of 3.7 and 8.02 when ovalbumin and BSA, respectively, were used as standards. The value of 5.71, which was similar to that found for keratin, decamin, and desmin (A450nm = 6.0; reference 13) was used throughout the present study.

Column Chromatography: A 25–35 mg protein aliquot (2–4 ml) of the urea-Tris solution was added to a column (90 × 2.5 cm) of Bio-Gel P-300 equilibrated against the same urea-Tris solution. The effluent was collected in 3.5-m1 fractions at a gravity flow rate of 6 ml/h (23°C) and monitored for protein at 280 nm. Protein (15–30 mg) from the major gel filtration peak (GFP) was added to a column of DEAE-Bio-Gel A (23 × 2 cm) in 8 mM urea-Tris in conjunction with step gradients of KCl (0–100 mM KCl) at 23°C. The effects of buffer (7.4–8.9) and KCl increments (5–10 mM) on resolution were examined.

Amino Acid Analyses: Amino acid analyses were done after 24, 48, and 72 h hydrolysis in 6.0 N HCl in vacuo after purging with nitrogen with a Glencoe MM amino acid analyzer (Glencoe Scientific Co., Houston, TX). Corroboratory analyses were obtained from AAA Laboratory (Mercer Island, WA) on a Dionex Corp. (Sunnyvale, CA) or Beckman Instruments (Fullerton, CA) Spino analyzer. Values for serine and threonine were extrapolated to zero time. The extent of modification was determined by assay of the carbohydrate content of hagfish polypeptide samples was monitored throughout the purification procedure by the phenolsulfuric acid method at 490 nm (15) in relation to d-glucose and d-galactose as standards. Hexosamines (glucosamine, galactosamine) were measured by column separation on the amino acid analyzer after hydrolysis in 4 N HCl at 100°C in evacuated test tubes. Discontinuous SDS-PAGE (22 ± 0.5°C) was used with a 7% resolving gel containing 7.8 M urea/20 mM Tris in conjunction with a 3% polyacrylamide upper gel also containing 7.8 M urea/20 mM Tris. The electrode buffer was prepared by avoiding stimulation of adjacent areas of pores. Care was taken to prevent possible contamination from gill slits by avoiding stimulation of adjacent pores.

RESULTS

Extraction of Polypeptides from GTCs: Comparison with Polypeptides in Whole Exudate and Vesicular Extract

We reported earlier (5) that the thread could be removed from the whole exudate (ruptured GTCs and GMCs) by tightly winking the fiber on a stirring rod and exhaustively washing the thread in ethanol-water solutions to provide a major polypeptide component with an M of ~63,500. This
The elution profile of the thiol derivatives of PE (heated at 60°C for 6 min, 10 mM DTT), from a DEAE-Bio-Gel A column, showed a major peak and at least five minor peaks at pH 8.0 (Fig. 3a) and a major peak and seven minor peaks at pH 8.9 (Fig. 3b). At either pH value, the major peak was obtained by exclusion from the column and usually represented 60–65% of the total material recovered. Resolution was either far less effective or not useful at pH values of <7.6.

Molecular weight evaluation of material in DEAE peaks 1–6 (Fig. 3a, pH 8.0) by SDS-PAGE (Fig. 4) showed that all components in peaks 1–5 (lanes b–e and h, respectively) had an Mr = 63,500 ± 500 daltons, but peak 6 contained a polypeptide of Mr ~67,000 and another of 58,000. Repetitive SDS-PAGE analyses of samples (~4–6 µg/lane) obtained from a given ion exchange preparation or from four independent ion exchange preparations at this pH produced similar results. SDS-PAGE analyses of DEAE fractions of PE obtained at pH 8.9 (Fig. 3b, peaks 1–6) were similar to DEAE peaks obtained at pH 8.0 (electrophoreograms not shown).

By means of discontinuous urea-PAGE analyses (Fig. 5) of PE and DEAE fractions, three major 63,500-dalton thread components were categorized based on the rate of electromigration to the anode. The α component in PE (lane M, arrow) migrated as a diffuse band with low mobility and under certain circumstances (e.g., prolonged contact with the electrode buffer prior to migration) did not enter the running gel (lane b). In contrast, the β and γ components migrated as sharper bands each with greater mobility than α. The component with greatest mobility to the anode was termed γ. DEAE peak 1 corresponded to the α component (lane n, entered gel; lane c,
and variable manner during urea-PAGE, appeared to exist in anion exchange chromatography and migrating in a diffuse 500. The a thread component, isolated by exclusion with a, ,B, and y thread polypeptides all have anM, of 63,500 t urea (23, 24). Unlike the keratins, which differed in M, the gel filtration or DEAE fractions ofa-keratin polypeptides in heteropolymer. A similar problem was encountered among existed as an aggregate in the form of either a homo- or 0, y) was made up of a unique polypeptide monomer or evaluated in the present report.

FIGURE 4 SDS-PAGE of thread polypeptides prepared by anion-exchange chromatography (Fig. 3a, pH 8.0) from precursor extract (PE). Lane a, precursor extract (PE) + DTT; lane b, DEAE-peak 1; lane c, DEAE-peak 2; lane d, DEAE-peak 3; lane e, DEAE-peak 4; lanes f and g, marker proteins corresponding to Fig. 1, lane e; lane h, DEAE-peak 5; lane i, DEAE-peak 6 containing components of ~67,000 (1, arrow) and 57,000 (2, arrow daltons; lane j, precursor extract (PE) with some high molecular weight aggregates evident; lane k, citraconic derivative of the a-component; lane l, carbamylate derivative of a-component; all samples heated with DTT. The major component in lanes a–e, h, and j has an M, of ~63,500 daltons.

did not enter gel). DEAE peak 2 represented the β component (lane d), peak 3 contained a mixture of β and γ (lane e), and peaks 4 and 5 both contained substances with the mobility of γ (lanes f and g). Hence, the relative amount of β to γ decreased progressively from peaks 2 to 5. Urea-PAGE analyses of DEAE fractions obtained at pH 8.9 (Fig. 3 b) yielded similar results (electrophoreograms are not shown, but categorization was recorded under absorbance peaks in Fig. 3 b), with the exception that the β and γ mixture was found in peak 4. The 67,000- and 58,000-dalton components (Fig. 4, lane i) and possible cationic proteins (22) were not further evaluated in the present report.

Of concern was whether each of the three components (α, β, γ) was made up of a unique polypeptide monomer or existed as an aggregate in the form of either a homo- or heteropolymer. A similar problem was encountered among gel filtration or DEAE fractions of α-keratin polypeptides in urea (23, 24). Unlike the keratins, which differed in M, the α, β, and γ thread polypeptides all have an M, of ~63,500 ± 500. The α thread component, isolated by exclusion with anion exchange chromatography and migrating in a diffuse and variable manner during urea-PAGE, appeared to exist in urea as non-covalently bound aggregates comprised of 63,500-dalton subunits. The discontinuous mode of urea-PAGE tended to promote aggregation, as judged by the immediate development of turbidity or precipitation upon admixing the upper buffer with PE or peak 1. None of the buffer systems tested (see Materials and Methods) prevented aggregation. However, two modes of chemical modification of the α component facilitated disaggregation and provided partial evidence that the α component can exist in urea as a homopolymer. By SDS-PAGE, the α-citraconic derivative(s) of the α component migrated as a single band with lower mobility than the unmodified α component (Fig. 4; lane k vs. b). The derivative had an M, of ~79,000, which is substantially higher than the expected value of ~65,000 if all the lysine groups were modified. Similar anomalous mobility and molecular weight estimates of anhydride-modified proteins have been reported and appear to be attributable to decreased SDS binding (25).

With urea-PAGE, however, the citraconic derivative(s) entered the gel and migrated as a single band with a mobility much higher than the unmodified α, β, and γ components (Fig. 5, lane j vs. components in all other lanes). The increased mobility to the anode is consistent with both the charge change expected by conversion of ε-amino groups to uncharged residues, as judged by the immediate turbidity or precipitation upon admixing the upper buffer with PE or peak 1. None of the buffer systems tested (see Materials and Methods) prevented aggregation. However, two modes of chemical modification of the α component facilitated disaggregation and provided partial evidence that the α component can exist in urea as a homopolymer. By SDS-PAGE, the α-citraconic derivative(s) of the α component migrated as a single band with lower mobility than the unmodified α component (Fig. 4; lane k vs. b). The derivative had an M, of ~79,000, which is substantially higher than the expected value of ~65,000 if all the lysine groups were modified. Similar anomalous mobility and molecular weight estimates of anhydride-modified proteins have been reported and appear to be attributable to decreased SDS binding (25).

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citramonic derivative of α-component in DEAE-peak 1; lane k, carbamylate derivative of α-component; lane l, same as b; lane m, PE + DTT in which α-component entered gel (arrow) but shows diffuse pattern; lane n, DEAE-peak 1, α-component aggregates entered gel. Note that lanes m and n were electrophoresed under somewhat different conditions than migrants in other lanes, and distance of migration is greater.

**TABLE I**

<table>
<thead>
<tr>
<th>Component separated by anion exchange chromatography</th>
<th>Mean pl, mean range of pl, and SEM of pl*</th>
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<tbody>
<tr>
<td>α*</td>
<td>7.56 (7.26–7.87) [0.08]</td>
</tr>
<tr>
<td>α-carbamylate derivative</td>
<td>5.99 (5.58–6.39) [0.02]</td>
</tr>
<tr>
<td>β</td>
<td>5.67 (5.52–5.81) [0.04]</td>
</tr>
<tr>
<td>γ</td>
<td>5.31 (5.18–5.47) [0.07]</td>
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*Isoelectric points were determined by reference to a pH gradient curve obtained by sectioning gel, eluting ampholytes, and measuring pH (25°C) with a combination microelectrode. 
†For a given run, the mean pl and the range of pl's of the isoelectric variants was obtained; after three runs of at least two samples of each component, the mean pl was calculated as was the mean range of pl's (in parentheses) and the standard error of the mean pl (in brackets).

**TABLE II**

<table>
<thead>
<tr>
<th>Amino Acid Composition of Hagfish Thread Polypeptides</th>
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<tr>
<td>Residues per 100 residues (mean + SEM)</td>
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<tr>
<td>α Component*</td>
</tr>
<tr>
<td>Asx 7.71 (0.36)</td>
</tr>
<tr>
<td>Thr 9.29 (0.18)</td>
</tr>
<tr>
<td>Ser 9.86 (0.20)</td>
</tr>
<tr>
<td>Glx 9.41 (0.17)</td>
</tr>
<tr>
<td>Pro 3.71 (0.40)</td>
</tr>
<tr>
<td>Gly 15.06 (0.43)</td>
</tr>
<tr>
<td>Ala 7.72 (0.09)</td>
</tr>
<tr>
<td>Val 7.00 (0.09)</td>
</tr>
<tr>
<td>Met 1.70 (0.32)</td>
</tr>
<tr>
<td>Ile 4.83 (0.12)</td>
</tr>
<tr>
<td>Leu 6.18 (0.21)</td>
</tr>
<tr>
<td>Tyr 3.40 (0.21)</td>
</tr>
<tr>
<td>Phe 2.16 (0.05)</td>
</tr>
<tr>
<td>His 2.05 (0.23)</td>
</tr>
<tr>
<td>Lys 3.30 (0.15)</td>
</tr>
<tr>
<td>Arg 4.89 (0.10)</td>
</tr>
<tr>
<td>Trp 0.67 (0.16)</td>
</tr>
<tr>
<td>Cys/2 0.22 (0.06)</td>
</tr>
</tbody>
</table>

*All values are based on at least three analyses of independently purified samples. Serine and threonine include corrections of 8% and 4%, respectively, to compensate for destruction during hydrolysis. Half-cystine was determined on separate samples as cysteic acid after performic acid oxidation. Tryptophan was determined separately by alkaline hydrolysis.

DEAE-prepared thread components were analyzed, but because of the aforementioned instability of other cyanate derivatives, the possibility of chemically induced artifacts is not ruled out. It should be emphasized that it was necessary to heat (for 5 min at 90°C with DTT, pH 8.0) the purified polypeptides to facilitate dissociation prior to electrophoretic analyses including isoelectric focusing. Under such conditions ~5% of lysine in these samples was converted to homocitrulline. Although no evidence of homocitrulline was seen when urea extracts of thread were stored frozen (−19°C) for 2–3 wk, some samples of purified α, β, and γ components were kept for longer intervals at this temperature in 5 M guanidine/20 mM Tris (pH 8.0) for subsequent self-assembly tests.

**Amino Acid Composition**

Although the amino acid composition (Table II) of α differed significantly from that of β and γ, each component was highest in Gly (~15.4 residues per 100) and relatively high in Glx, Ser, Thr, Asx, Ala, Val, and Leu, but relatively low in Cys/2 and Trp. Relative to the amino acid content of β and γ, it appeared that α was higher in Pro, Tyr, His, and Trp, but lower in Glx, Met, and Leu. In contrast, the compositions of β and γ were very similar to each other. Hence, these
results further support the view that the α component represents a unique polypeptide chain, which may form a homopolymer but does not form a heteropolymer with β or γ components under conditions employed for DEAE chromatography. In view of the aforedescribed differences in amino acid and carbohydrate contents, and the observation by SDS-PAGE that each of the components is comprised of 63,500-dalton polypeptides, it is unlikely that the α component is either a precursor or degradation polypeptide of β or γ.

Filament Formation In Vitro

Mixtures of the thiol forms of α, β, and γ components, which were purified by anion exchange, assembled into 9–11-nm filaments when dialyzed for 18 h against 5 mM Tris (pH 8.0–8.9) (Fig. 6, a–d). The filaments were sometimes highly tangled and twisted (Fig. 6 a), but single and relatively straight IFs were discerned (Fig. 6, b–d) with cross-sectional diameters within the expected 9–11-nm range for reassembled and negatively stained specimens. Among IF reassemblies reported heretofore, both tangled aggregates (28, 29) and smooth-edged, curvilinear (23, 30) filaments have been imaged after negative staining. Although reconstitution of IFs was best obtained with mixtures of α, β, and γ, which is indicative of a copolymerization process, the α component alone sometimes formed macrofibrils consisting of loosely

![Figure 6](https://example.com/figure6.png)

**Figure 6** Self-assembly in vitro of purified thread polypeptides. (a) Mixture of α, β, and γ at a ratio of 1:1:1 dialyzed against 5 mM Tris buffer (pH 8.9); highly tangled 9–11-nm filaments are mainly evident. (b–d) Mixture of α, β, and γ (1:1:1) dialyzed against 5 mM Tris buffer (pH 8.2) and then diluted with the buffer to facilitate localization of individual 9–11-nm filaments; negatively stained with uranyl acetate. Bar, 100 nm. × 128,000.
aligned filaments, often with cross-sectional diameters of ~6 nm (data not shown).

DISCUSSION

This investigation provides a procedure for the isolation and characterization of IF polypeptides derived from hagfish GTCs. The GTC is unique in its ability to synthesize and assemble numerous IFs into parallel alignment for the purpose of forming a single, tightly coiled thread that is compartmentalized into 60-80% of the cell volume at maturity. At this stage, the resultant tapered thread may have attained a length of ~60 cm and a cross-sectional diameter of 0.5-1.5 μm (5). Some of the morphological changes that accompany maturation of the thread, including the development and disappearance of interspersed microtubules, are described elsewhere (8, 9).

The urea-Dulbecco’s cycled extract (PE), obtained from a purified population of GTCs, yielded three 63,500-dalton components (α, β, and γ) by ion exchange chromatography. Abundant evidence was available to suggest that each of the three components corresponded to substances in thread IF. GTCs are mainly composed of thread (5); the bulk of thread material consists mainly of IFs (9); >90% of the PE is composed of α, β, and γ (area under curves in Fig. 3, a and b); a mixture of the three polypeptides assembles in vitro to 9-11-nm filaments (Fig. 6, a-d); each of the three components is antigenically related to thread material (indirect immunofluorescence, data not shown); and PE, α, β, and γ have SDS-PAGE mobilities different from substances extracted from the other major gland cell type, the GMC (Figs. 1 and 4).

Our finding of an M, of 63,500 for each of the α, β, and γ thread components in Pacific hagfish (Eptatretus stouti) was not in agreement with the value of 14,000-18,000 reported by Hashimoto et al. (11), as cited by Terakado et al. (12) for a fibrous component from the slime glands of Japanese hagfishes (Paramyxine atami and Eptatretus burgeri). Inasmuch as their mode of purification was not described and self-assembly was not tested, it is not possible at present to explain the disparate values. Although a species difference may be one explanation, an M, of 14,000-18,000 is markedly below the lowest value of ~40,000, which has been reported to date for IF polypeptides from various sources (30, 32-34). Moreover, with Eptatretus stouti, we have used other isolation procedures which confirm that the thread material is exceedingly rich in 63,500-dalton polypeptides; e.g., a guanidine-Tris extract that was cycled with Dulbecco’s solution (Fig. 1, lane b); the thread material was physically removed and exhaustively washed in alcohol-water solutions and then extracted with urea and 2-mercaptoethanol (5).

Several lines of evidence corroborated the view that the thiol forms of α, β, and γ components each consisted of a unique 63,500-dalton polypeptide, which, in the case of α, could exist as a homopolymer in 8 M urea-Tris solutions. The major component α differed significantly in amino acid composition and pl from both β and γ. In contrast, β and γ were very similar to each another in amino acid content but differed in mean pl values (5.67 vs. 5.31, respectively, Table I). The high pl of α (7.56) may be attributable in part to a lower content of Glx (Glu) relative to β or γ (Table II). The small difference in pl between β and γ may be due in part to phosphorylation differences, isoelectric variants as artifacts accompanying purification, and naturally occurring isoelectric variants that exist in a population of pooled GTCs obtained at varying developmental stages. The significance of a possible carbohydrate moiety associated only with β, as inferred by analysis with the phenol-sulfuric acid reagent (Fig. 3 b, upper curve) is unclear at present. Although IFs from many sources appear to be devoid of carbohydrate (30), evidence of glycosylation among keratin IFs in the MEO cell line has been reported (31).

The amino acid compositions of α, β, and γ thread polypeptides of the epidermally derived slime glands bear a similarity to some keratin polypeptides. The three thread components (Table II) are relatively rich in Gly, Glx, and total hydroxyamino acids (15.1-15.9, 9.4-12.8, and 18.6-19.2%, respectively). Comparisons with bovine epidermal keratin (24), keratins derived from stratum corneum of human (35) and rat (36) origin, and IF polypeptides of bovine esophageal epithelium (37) show for the same amino acids, respectively, values of 12.5-17.2, 10.6-16.1, and 13.7-18.3%. In contrast, IFs from other cell types show a lower content of Gly and hydroxyamino acids, respectively: chicken gizzard desmin (6.1 and 12.3% [28]), pig eye vimentin (2.7 and 15.1% [28]), keratin (5.0 and 13.0% [38]), calf brain neurofilament protein (6.0 and 8.2% [39]), and human astrocyte filaments (4.9 and 10.6% [40]). In common with IF components isolated from many sources (23, 28, 35, 36-39, 41), thread polypeptides were high in Glx but low in Trp and Cys/2. Preliminary studies reveal that each of the three hagfish thread polypeptides bears an antigenic similarity to human keratins. Antigenic similarity of thread polypeptides to IFs from other vertebrates is under investigation.

Of possible usefulness was the observation that the thiol forms of the α aggregates or α homopolymers in urea solution reacted with cyanate or citraconic anhydride to form polypeptide derivatives which migrated with increased mobility to the anode and increased homogeneity through the 7% resolving gel during urea-PAGE. In marked contrast, the unmodified α material exhibited variable and anomalous electrophoretic characteristics, sometimes not penetrating the 3% upper gel, occasionally entering this gel but forming threadlike aggregates therein, or often entering the lower gel in an uneven manner (e.g., Fig. 3, lanes m and n). On the basis of the reaction conditions used and the properties of the derivatives that were observed, it is likely that the lysine residues were modified (20, 21, 26) in a manner that also facilitated disaggregation of the α polypeptides. Still to be ascertained is whether the derivatives might inhibit the 9-11-nm self-assembly process and therefore provide a means to implicate specific amino acid residues in stabilization of IFs.

Whether the α, β, and γ polypeptides represent, singly or collectively, the principal components for in vivo assembly in the form of three chain models (42) or other proposed models (43, 44), or correspond to a differential expression of IF polypeptides among the mixed-aged population of GTCs used as a source, remains to be elucidated. Among the thread polypeptides isolated by anion exchange was a minor component with an M, of 67,000. The role of this polypeptide or its relationship to the “ubiquitous” 66,000-dalton component of the type described by Pruss et al. (45) is not known. Of interest are recent reports (13, 22, 46) of a species-specific cationic structural protein termed filaggrin (13), which interacts with IFs to form large fibers (macrofibrils) and induces parallel alignment of IFs. None of the purified hagfish thread
polypeptides show a high content of histidine and arginine or a high pi of $\geq$10. However, the urea-Dulbecco’s cycling procedure was highly effective in yielding an extract rich in IF components but poor in retaining other polypeptides; hence, a filaggrin-like component in GTCs cannot be ruled out.

The finding that reassembly of 9–11-nm filaments is best achieved with mixtures of the $\alpha$, $\beta$, and $\gamma$ thread components may be indicative of obligative copolymers, as is characteristic of keratin IF (30, 47). On the basis of our in situ observations on the growth (thickening and lengthening) of the IF-rich gland thread (8, 9), the IF polypeptides might be expected to show novel aggregates in vitro. Preliminary studies show that each of the three polypeptides ($\alpha$, $\beta$, $\gamma$) has a multipotential ability to assemble into different filamentous forms and macrofibrils (48).

Specific cellular functions of IFs are still mainly speculative (33), but in the case of the hagfish the IF-rich thread may modulate the viscoelastic properties of mucus that is formed after release of GTCs and GMCs by holocrine secretion. Whether the high content of hydroxyamino acids of the thread polypeptides provides sites for interaction with vesicular glycopolypeptides remains to be evaluated.

Ferry, writing in 1941 (10), was among the earliest investigators to record some properties of the fibrous product of the hagfish slime gland and chose to term the substance “minitin” ($\mu$trous Gr., thread). Inasmuch as the fibrous thread was found in our investigation to be composed mainly of IFs, the word minitin appears appropriate to designate IFs derived from thread of hagfish GTCs. It remains to be seen if thread cells located within the epidermis (2, 4) but external to the slime glands have similar IF polypeptides.

On the basis of the present and recent reports (7–9), the hagfish gland thread cells, which function mainly to form massive quantities of IFs in a precisely compartmentalized manner, offer a unique and potentially useful system to evaluate factors responsible for in vitro and in vivo assembly of filamentous systems and for interactions with one another.

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