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Biochemistry

Characterization of a class of cationic proteins that specifically interact with intermediate filaments

(10-nm filaments/filaggrin/macrofibrils/protein polymerization)

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ABSTRACT **We describe a class of cationic structural proteins that associate specifically with intermediate filaments (IF) but not with other types of cytoskeletal proteins. These proteins, for which the term *filaggrin* is introduced, are isolated from the stratum corneum of mammalian epidermis. They are species-distinct proteins; for example, rat and mouse filaggrin have different molecular weights and amino acid compositions, but are nevertheless chemically and functionally very similar. They interact *in vitro* with the IF of several different types of cells to form large fibers or macrofibrils in which many IF are highly aligned in parallel arrays. Stoichiometric analyses suggest that two molecules of filaggrin bind to each three-chain building block of the IF, possibly by ionic interactions with the coiled-coil α-helical regions of the IF.**

Intermediate filaments (IF) are ubiquitous constituents of the cytoskeleton of eukaryote cells. However, detailed studies of the IF isolated from a wide variety of different types of cells have indicated broad differences in their solubility and immunological properties and in the size and complexity of their constituent subunits (1–3). Nevertheless, the IF studied to date appear to be structurally analogous. They are all α-type fibrous proteins and are composed of a common three-chain building block (4–7).

Mammalian epidermis is unusual in that its principal differentiation products are filaments of the IF type (keratin filaments). These are synthesized and deposited intracellularly in the inner living cell layers as bundles or fibrils and eventually form the bulk of the terminally differentiated cells of the stratum corneum (8). The epidermis also produces significant amounts of another structural protein, which has distinctly different physicochemical properties from the IF. This protein is synthesized in the granular layer as a highly phosphorylated precursor and accumulates in amorphous keratohyalin granules, but it is dephosphorylated to become highly cationic when the granular cells differentiate to form the stratum corneum cells (9). The rat cationic protein, called stratum corneum basic protein (10), histidine-rich protein II (11), or histidine-rich basic protein (12), is thought to function in the stratum corneum as an interfilamentous matrix and thereby contribute to the formation of the characteristically insoluble protein complex of the epidermis, keratin. Support for this notion has been adduced from experiments *in vitro*. Mixing of epidermal keratin IF with the rat cationic protein results in the formation of insoluble fibrous structures, or macrofibrils (12–15). These macrofibrils consist of large numbers of keratin IF aligned in parallel arrays reminiscent of the keratin fibrils in the stratum corneum.

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In this paper, we report the isolation and characterization of a functionally similar although chemically distinct protein from mouse epidermis. Both the rat and mouse cationic proteins form macrofibrils when mixed with epidermal and inner root sheath keratin IF and also with IF repolymerized from fibroblastic (vimentin = decamin) and muscle (skeletin = desmin) cells. We conclude that the rat and mouse cationic proteins, for which the term *filaggrin*[§] is introduced, represent two members of a class of proteins that function in cells by their specific interaction with IF.

MATERIALS AND METHODS

Isolation of Mouse Filaggrin. Newborn mouse epidermis was prepared by flotation on trypsin and the stratum corneum was collected as a retentate after removal of the viable epidermal cells by filtration (17). The stratum corneum was washed twice in phosphate-buffered saline containing 1 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$) and then extracted with a buffer of 8 M urea/0.1 M Tris·HCl, pH 7.4/0.1 M 2-mercaptoethanol/0.5 mM $PhMeSO_2F$ for 1 hr, homogenized in a Brinkmann Polytron, and extracted for a further 2 hr. The extract contains filaggrin as well as the bulk of the keratin IF in the form of soluble subunits. The filaggrin was recovered in an enriched form by exclusion chromatography on DEAE-cellulose in a buffer of 8 M urea/10 mM Tris·HCl, pH 7.4/1 mM dithiothreitol/0.5 mM $PhMeSO_2F$ and then purified to homogeneity by preparative $NaDodSO_4$ gel electrophoresis (18). After removal of the detergent by ion-pair extraction (19), the filaggrin was recovered freeze-dried in its formate form by frontal elution chromatography from a column of Bio-Gel P-6 (Bio-Rad) equilibrated in 8.8% (wt/vol) formic acid.

Other Basic Proteins. Rat filaggrin was prepared as described (10, 15). Calf thymus histones (mixed type II-S, lysine-rich type III-S, and arginine-rich type VIII-S) were obtained from Sigma.

Preparation of IF and Other Fibrous Proteins. Epidermal keratin IF subunits from newborn mouse epidermal tissue were harvested from the above DEAE-cellulose column by elution with 0.1 M KCl (20). These subunits as well as a mixture of bovine epidermal subunits 3 and 5 were then assembled into native-type IF *in vitro* by standard procedures (21). Inner root sheath keratin IF were isolated as morphologically-intact fila-

Abbreviations: IF, intermediate filament(s); $PhMeSO_2F$, phenylmethylsulfonyl fluoride.

§ We propose this term (fĭl-ăg′-grĭn) to describe the unique function of these proteins in the specific aggregation of intermediate filaments. The name *stratum corneum basic protein* (10) was meant to be used temporarily until the function of the proteins was understood. While these proteins contain a modest amount of histidine (≈8%), more than most proteins, they are not histidine-rich in comparison to certain other proteins (16).

ments (22). Fibroblastic IF were assembled *in vitro* from decamin isolated and purified from BHK-21 hamster cells (7, 19). Muscle IF were likewise prepared *in vitro* from the α and β desmin subunits of both BHK-21 cells and of hamster stomach smooth muscle desmin (19). F-actin was polymerized *in vitro* from actin isolated from mouse epidermal cells grown in culture (23). Chicken brain tubulin was purified by three cycles of assembly–disassembly *in vitro* (24).

Analytical Procedures. Protein was estimated spectrophotometrically: keratin, decamin, and desmin each had $A_{277}^{1\%} = 6.0$; and the filaggrin preparations had $A_{277}^{1\%} \approx 1.4$ Analytical polyacrylamide gel electrophoresis with 0.1% $NaDodSO_4$ was done on 1.5-mm-thick slab gels with a 10–18% acrylamide gradient (6). Amino acid analysis was done after 22-, 48-, and 72-hr hydrolyses in 5.7 M HCl at 105°C on a Beckman 119CL amino acid analyzer equipped with a model 126 data reduction system. Amino-terminal residues were identified by reaction with dansyl chloride in 0.1 M NH_4HCO_3/1% $NaDodSO_4$ (25). Carboxyl-terminal amino acids were released by reaction with a mixture of carboxypeptidases A and B (Sigma) and identified by amino acid analysis (25). Circular dichroitic spectra were determined by established procedures (6).

Ultracentrifugation. Solutions of mouse or rat filaggrin (0.6 mg/ml) were dialyzed against 100 vol of 6 M guanidine hydrochloride for 48 hr and then examined by sedimentation equilibrium in the Beckman analytical ultracentrifuge at 32,000 rpm as described (26). Values for ϕ', the apparent specific volume, were calculated from the amino acid composition by the method of Lee and Timasheff (27).

Immunologic Studies. Crossreaction between the rat and mouse proteins was tested by Ouchterlony double diffusion and by the gel transfer method (28). The proteins were separated by electrophoresis on $NaDodSO_4$/polyacrylamide slab gels containing *N,N'*-diallyltartardiamide crosslinker. The gel was treated with periodic acid and then sandwiched between two sheets of diazobenzyloxymethyl-paper for transfer of proteins (Transa-Bind, Schleicher & Schuell). The paper was incubated with antiserum to rat filaggrin or preimmune control serum. After washing with four changes of buffer I (28), the paper was incubated with 0.25 μCi (1 Ci = 3.7×10^{10} becquerels) of ^{125}I-labeled staphylococcal protein A per gel slot for 2 hr, washed, dried, and fluorographed.

Macrofibril Formation *In Vitro*. Mouse or rat filaggrin was equilibrated in a buffer compatible with the IF type to be studied: 5 mM Tris·HCl, pH 7.4/0.1 mM $PhMeSO_2F$ for keratin or inner root sheath IF and 5 mM Tris·HCl, pH 7.4/0.17 M NaCl/0.1 mM $PhMeSO_2F$ for decamin or desmin IF. Subsequently, the two components were mixed at the desired protein concentration and molar ratio. In stoichiometry studies, macrofibrils were pelleted at $2000 \times g$ for 5 min. Under these conditions, solutions of filaggrin or the IF alone did not sediment. Samples of mixtures and pellets of macrofibrils were dispersed into gel electrophoresis buffer containing $NaDodSO_4$ and analyzed on 6-mm 10% polyacrylamide tube gels for quantitation of the two components by scanning densitometry (21).

Electron Microscopy. Negative staining was done by using 0.7% uranyl acetate (21). Pellets of macrofibrils were fixed and sectioned by standard procedures (13).

RESULTS

Isolation and Purification of Mouse Filaggrin. Extraction of newborn mouse epidermal stratum corneum with a denaturing solvent releases about 95% of the tissue (by weight) as soluble protein. Of this, the keratin IF subunits (K_1 and K_2 families of $M_r \approx 68,000$ and $\approx 60,000$, respectively), represent about 60%, and much of the remainder is filaggrin, of $M_r \approx 30,000$ (Fig. 1, lane a). Owing to its strongly cationic charge, filaggrin was easily separated from the anionic keratin subunits by exclusion chromatography on DEAE-cellulose (Fig. 1. lane b) and subsequently purified to homogeneity by preparative $NaDodSO_4$ gel electrophoresis (Fig. 1, lane c). The yield was about 20 mg/g (dry weight) of stratum corneum. The rat filaggrin has $M_r \approx 48,000$ (Fig. 1, lane d; ref. 10).

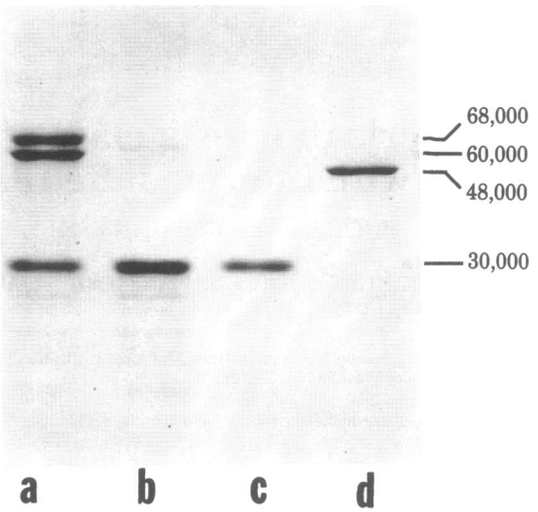

FIG. 1. Gel electrophoresis of filaggrin. Lane a, proteins extracted from newborn mouse stratum corneum consist primarily of the K_1 ($M_r \approx 68,000$) and K_2 ($M_r \approx 60,000$) IF subunits as well as filaggrin ($M_r \approx 30,000$); lane b, enrichment of filaggrin after exclusion from DEAE-cellulose; lane c, pure mouse filaggrin after preparative gel electrophoresis; lane d, rat filaggrin of $M_r \approx 48,000$ prepared as described (10).

Properties of Mouse and Rat Filaggrin. Preliminary sedimentation equilibrium ultracentrifugation experiments indicated that in aqueous solutions, such as phosphate-buffered saline, mouse filaggrin had a high and heterogeneous molecular weight; that is, it was aggregated. In the presence of 6 M guanidine hydrochloride, however, it behaved essentially as a single species of molecular weight 25,840 ± 170 (Fig. 2). A similar analysis of rat filaggrin gave a molecular weight of 38,400. The discrepancy in molecular weight estimates between sedimentation equilibrium and $NaDodSO_4$ gel electrophoresis may be due to the anomalous interactions of the strongly cationic protein with the detergent.

The pI of rat and mouse filaggrin exceeded the upper pH limit of all commercially available isoelectric focusing media and was thus >10. The amino acid compositions (Table 1) demonstrate the presence of large amounts of Ser, Glx, Gly, Ala, His, and Arg and none or only trace amounts of Cys, Met, Lys, and Trp. While generally similar to each other, rat and mouse filaggrin are significantly different with respect to their contents of Thr, Val, Ile, Leu, Tyr, and Phe (Table 1). End group analyses of both proteins indicated a blocked aminoterminus and arginine at the carboxylterminus. By circular dichroism, the proteins contained 10–15% α-helix and 20–25% β-helix.

The rat and mouse filaggrin are immunologically related as shown by reaction with antiserum to the rat protein (Fig. 3). However, mouse filaggrin did not form a precipitin line with this antiserum in double-diffusion analysis.

Macrofibril Formation *in Vitro*. Upon mixing either mouse or rat filaggrin with all types of IF investigated, there was a very rapid (within 1–5 sec) increase in turbidity, flocculant fiber (macrofibril) formation, or both. The exact nature of the reaction depended on the relative amounts of the two components mixed and on the total protein concentration. Macroscopic fibrous ag-

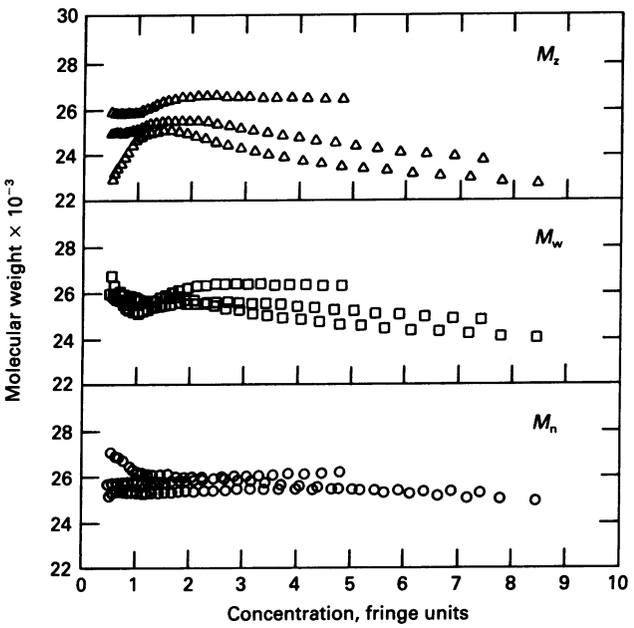

FIG. 2. Molecular weight distribution of filaggrin obtained by equilibrium sedimentation. Plots of molecular weight vs. concentration (fringes) from analysis of Rayleigh plates were generated by computer. Initial loadings were approximately 0.2, 0.4, and 0.6 mg/ml. Pictures were taken between 21 and 23 hr. M_1 = 25,840 ± 170; M_n = 25,890 ± 70; M_w = 26,100 ± 120; M_z = 25,820 ± 90. M_1 represents the smallest molecular weight species (26). M_n, M_w, and M_z represent the number-average, the weight-average, and the *z*-average molecular weights, respectively, extrapolated to 0 concentration. Apparent specific volume (in 6 M guanidine hydrochloride): 0.686 ml/g; ± represents SEM.

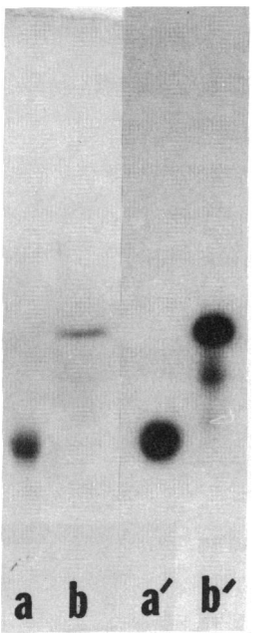

FIG. 3. Reaction of filaggrin with antiserum to the rat filaggrin. (*Left*) $NaDodSO_4$ gel electrophoresis of mouse filaggrin, lane a, and rat filaggrin, lane b. (*Right*) Fluorograph of the reaction of the these proteins transferred to paper and incubated with antiserum to rat filaggrin, then with ^{125}I-labeled staphylococcal protein A: mouse protein, lane a′; rat protein, lane b′. The lower molecular weight bands in the rat filaggrin are degradation products formed during storage.

gregates that rapidly precipitated from solution resulted when the total protein concentration exceeded about 1 mg/ml. Many macrofibrils were too large to attach to the electron microscope grid. In mixtures containing limiting amounts of IF, the macrofibrils appeared as imperviously dense fibrils that were more than 1 μm wide and many μm long (Fig. 4*a*). In mixtures containing limiting amounts of filaggrin, the macrofibrils were less compact (Fig. 4*b*). In thin sections, the dense macrofibrils consisted of very large numbers (several hundred or more) of IF that were aligned in parallel arrays. In longitudinal section, the IF appeared as lighter-stained circles or annuli 7–9 nm wide (Fig. 4*d*); in both sections, the IF were separated by 2–4 nm of darker-stained material, presumably filaggrin. This image formed in macrofibrils of all IF types used and is reminiscent of the keratin pattern seen in the stratum corneum of the epidermis (8).

Table 1. Amino acid composition (mol/100 mol) of filaggrin

Amino acid	Mouse	Rat (ref. 10)
Aspartic acid	5.2	3.6
Threonine	0.4	5.7
Serine	20.4	17.3
Glutamic acid	20.6	20.5
Proline	3.1	2.8
Glycine	17.0	14.4
Alanine	8.6	11.8
Cystine (half)	0.1	0
Valine	3.0	0.6
Methionine	0.1	0
Leucine	0.7	0
Isoleucine	0.2	1.4
Tyrosine	0.6	0.1
Phenylalanine	0.6	0
Histidine	8.4	7.9
Lysine	0.1	0
Tryptophan	0	0
Arginine	11.5	13.9

The macrofibrils formed at total protein concentrations of 0.3–0.5 mg/ml assumed a turbid suspension, and on negative staining appeared as large, highly folded, looped, and convoluted structures that consisted of distinct fibers 50–100 nm wide (Fig. 5). Each fiber contained 5–20 individual IF aligned side by side in either "tight" (Fig. 5*a*) or "loose" (Fig. 5*b*) parallel arrays, depending on whether the relative amount of IF was limiting or in excess, respectively. At low total protein concentrations (<0.1 mg/ml), the macrofibrils were smaller loosely bound structures that were only two to five IF wide (Fig. 5*c*). IF exist in equilibrium with their constituent protofilaments (21, 29). At protein concentrations just above the critical concentration of assembly, 50–100 μg/ml, large numbers of protofilaments are also present in the IF preparations. From Fig. 5*c* it is apparent that the protofilaments are not ordered into large structures by the filaggrin.

Two types of control experiments were performed. Mixtures of rat or mouse filaggrin with F-actin or microtubules at protein concentrations ranging between 0.1 and 2 mg/ml resulted in a slight increase in the turbidity of the solution. By negative staining, the anionic filaments appeared randomly covered by the cationic filaggrin (see ref. 14). Mixtures of histones with the IF types resulted in a distinct increase in turbidity that, even at the highest protein concentrations, could not be clarified by centrifugation at 2000 × *g*. By negative staining, structures containing two to four IF were often seen arranged in elongated arrays but not into macrofibrils (data not shown). Histones also associated with F-actin and microtubules in a similarly irregular manner.

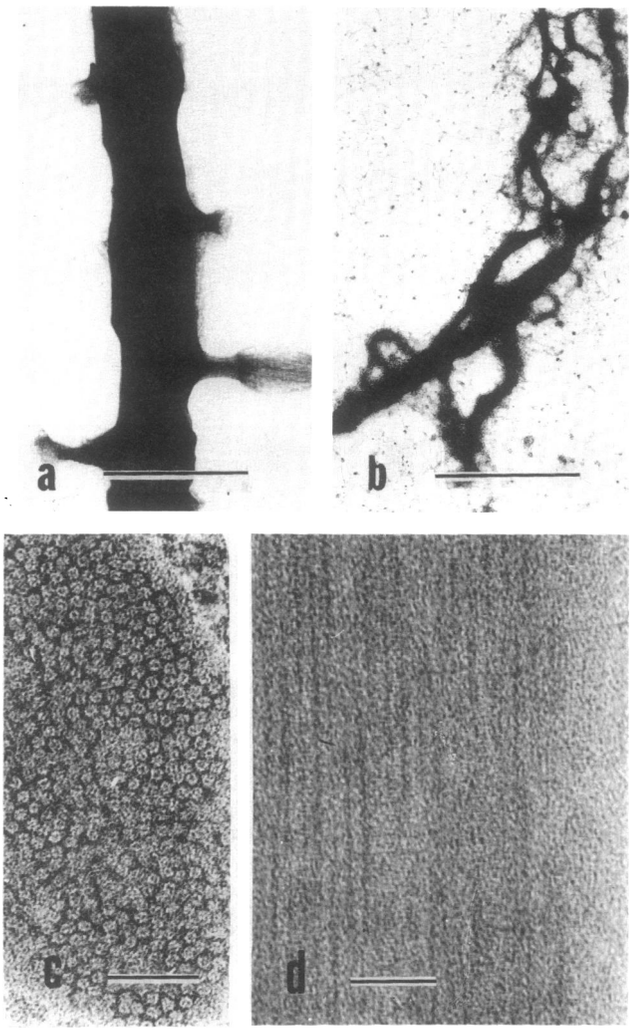

FIG. 4. Macrofibrils formed at high protein concentration. Macrofibrils were formed by mixing approximately equal volumes of: (*a*) mouse filaggrin (1 mg/ml) and mouse epidermal keratin IF (≈2 mg/ml) (molar ratio ≈1:1) in 5 mM Tris·HCl, pH 7.4/0.1 mM $PhMeSO_2F$; (*b*) mouse filaggrin (0.3 mg/ml) and decamin (1.8 mg/ml) (molar ratio ≈1:3) in 5 mM Tris·HCl, pH 7.4/0.17 M NaCl/0.1 mM $PhMeSO_2F$. (Negatively stained with uranyl acetate; ×19,000; the bar represents 1.0 μm.) Macrofibrils of sample *a* were sectioned in transverse (*c*) and longitudinal (*d*) direction. (×213,000; the bar represents 50 nm.)

Stoichiometry of Macrofibril Formation. Mouse filaggrin was mixed with mouse epidermal keratin and desmin IF in various relative molar amounts, and, interestingly, the resulting macrofibrils contained the two components in invariant amounts (Fig. 6) of 39–44% filaggrin and 56–61% IF; that is, about 40% and 60%, or about 2 mol of filaggrin per 3 mol of IF subunits. Similar stoichiometric patterns were evident in macrofibrils formed with rat filaggrin and with bovine epidermal keratin and decamin IF.

DISCUSSION

The data presented in this paper suggest that mouse filaggrin is a homogeneous protein. It is apparently identical to a protein isolated by Balmain *et al.* (30). In addition, mouse and rat filaggrin are biochemically and immunologically similar but chemically distinct cationic proteins. Preliminary experiments indicate that immunologically similar proteins exist in the stratum corneum of other species (unpublished data). Thus we conclude that filaggrins are a family of similar but species-distinct proteins.

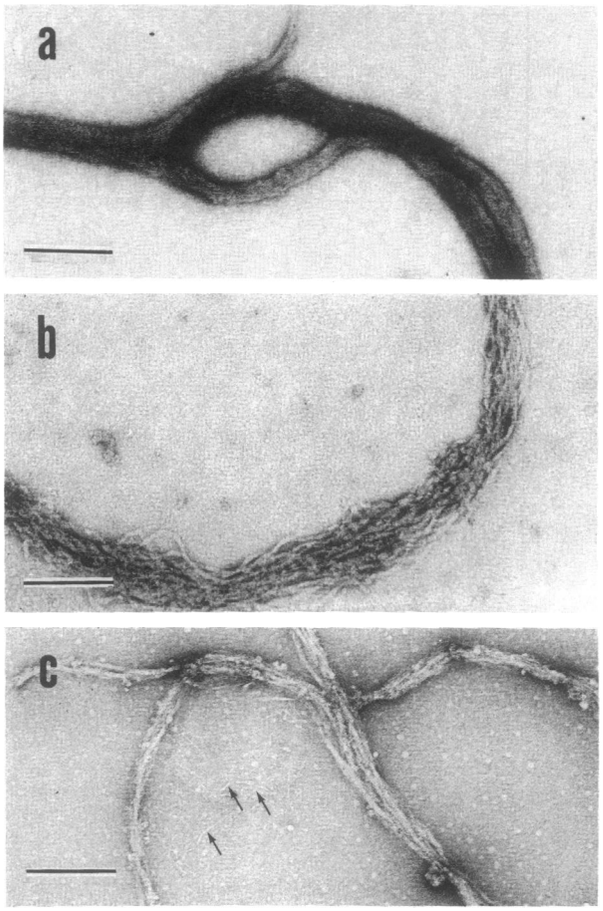

FIG. 5. Macrofibrils formed at intermediate and low protein concentrations. Macrofibrils were formed by mixing approximately equal volumes of: (*a*) mouse filaggrin (0.2 mg/ml) and BHK α-desmin IF (0.4 mg/ml) (molar ratio ≈1:1); (*b*) rat filaggrin (0.2 mg/ml) and bovine epidermal keratin IF (0.6 mg/ml) (molar ratio ≈1:6); (*c*) mouse filaggrin (≈30 μg/ml) and hamster smooth muscle desmin (mixture of α and β subunits, ≈60 μg/ml) (molar ratio ≈1:1); protofibrils are indicated by the arrows. (Negatively stained with uranyl acetate; ×55,000; the bar represents 0.2 μm.)

We demonstrate here that rat and mouse filaggrin align epidermal and inner root sheath keratins, as well as muscle and fibroblastic IF, into highly ordered linear arrays, or macrofibrils. In preliminary experiments, mouse filaggrin similarly aggregates squid neurofilaments (unpublished data). That is, filaggrin interacts with four of the five subgroups of IF (2, 3), and in view of the similarities of all IF, we predict it will interact with glial IF as well. However, filaggrin does not interact to form ordered structures with several other types of fibrous proteins (13, 14) including F-actin and microtubules, which are also prominent cytoskeletal proteins present in eukaryote cells.

In view of the apparent high degree of specificity, it would seem likely that filaggrin interacts with sites or structural domains that are found only in IF. The IF of different cells possess distinctly different immunological, chemical, and solubility properties (1–3), yet those studied in detail, and probably all IF, are composed of a similar three-chain structural building block that contains two regions of coiled-coil α-helix interspersed by regions of non-α-helix (6). The observed stoichiometry of association of about 2 mol of filaggrin with 3 mol of IF subunits is of interest in this regard; it is possible that a cationic filaggrin molecule binds each anionic α-helical coiled-coil region of the IF. However, further experiments are required to understand the mechanism of interaction of the proteins.

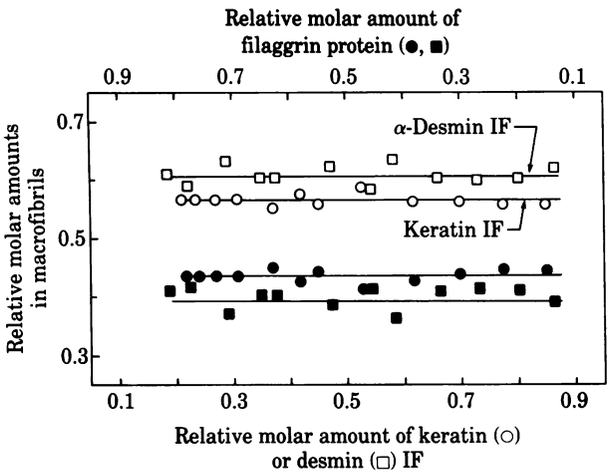

FIG. 6. Stoichiometry of macrofibril formation. Solutions of mouse epidermal keratin or BHK α-desmin IF and mouse filaggrin, equilibrated in the salt solution compatible with the IF type, were adjusted to an exactly known protein concentration (≈1 mg/ml). Salt solution was added to IF samples before addition of the filaggrin so that for each of 12 mixtures the final volume was about 0.5 ml; protein concentration was about 0.4 mg/ml; and the molar ratio of the two components in the mixtures varied between about 1:5 and 5:1. After standing for 10 min at 23°C, the mixtures containing turbid or precipitated macrofibrils were mixed vigorously. Aliquots were withdrawn and the remainder was pelleted at 2000 × *g* for 5 min for quantitation of the two components by gel electrophoresis. The molecular weights used for calculation of molar amounts were: mouse filaggrin, 26,000; desmin, 54,000; and mouse epidermal keratin, 64,000 (average of the two subunits).

To date, most of the available chemical and structural information on IF of different cells has been acquired by solution protein chemical techniques and, notably, by the *in vitro* assembly–disassembly paradigm. Although this approach has been invaluable in the identification of the proteins that are integral subunits of the IF, it suffers from the potential disadvantage that IF-associated proteins, which may play an important role in the functions of IF in cells, may be lost. The filaggrin species described here are a class of proteins that specifically interact with IF. The distribution of chemically or functionally similar cationic proteins in cells other than epidermal keratinocytes remains to be investigated.

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1. Goldman, R. D., Milsted, A., Schloss, J. A., Starger, J. M. & Yerna, M.-J. (1979) *Annu. Rev. Physiol.* **41**, 703–722.
2. Lazarides, E. (1980) *Nature (London)* **283**, 249–256.
3. Zackroff, R. V., Steinert, P. M., Anyardi-Whitman, M. & Goldman, R. D. (1981) *Cell Surface Reviews*, ed. Hartshorne, D. (North-Holland, Amsterdam), Vol. 7, in press.
4. Day, W. A. & Gilbert, D. S. (1972) *Biochim. Biophys. Acta* **285**, 503–506.
5. Steinert, P. M., Zimmerman, S. B., Starger, J. A. & Goldman, R. D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6098–6101.
6. Steinert, P. M., Idler, W. W. & Goldman, R. D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4534–4538.
7. Cabral, F., Zimmerman, S. B., Gottesman, M. M. & Steinert, P. M. (1981) *J. Biol. Chem.* **256**, 1428–1431.
8. Fraser, R. D. B., MacRae, T. P. & Rogers, G. E. (1972) *Keratins, Their Composition, Structure and Biosynthesis* (Thomas, Springfield, IL), pp. 150–159.
9. Lonsdale-Eccles, J. D., Haugen, J. A. & Dale, B. A. (1980) *J. Biol. Chem.* **255**, 2235–2238.
10. Dale, B. A. (1977) *Biochim. Biophys. Acta* **491**, 193–204.
11. Ball, R. D., Walker, G. K. & Bernstein, I. A. (1978) *J. Biol. Chem.* **264**, 5861–5868.
12. Dale, B. A., Vadlamudi, B., DeLap, L. W. & Bernstein, I. A. (1981) *Biochim. Biophys. Acta* **668**, 98–106.
13. Dale, B. A., Holbrook, K. A. & Steinert, P. M. (1978) *Nature (London)* **276**, 729–731.
14. Steinert, P. M., Dale, B. A. & Holbrook, K. A. (1980) in *Fibrous Proteins: Scientific, Industrial and Medical Aspects*, Part 2, eds. Parry, D. A. D. & Creamer, L. K. (Academic, London), pp. 177–184.
15. Dale, B. A., Lonsdale-Eccles, J. D. & Holbrook, K. A. (1980) in *Biochemistry of Normal and Abnormal Epidermal Differentiation*, eds. Bernstein, I. A. & Seiji, M. (Tokyo Univ. Press, Tokyo, Japan), pp. 311–325.
16. Kilejian, A. (1974) *J. Biol. Chem.* **249**, 4650–4655.
17. Yuspa, S. H. & Harris, C. C. (1974) *Exp. Cell Res.* **86**, 95–105.
18. Steinert, P. M. & Idler, W. W. (1975) *Biochem. J.* **151**, 603–614.
19. Steinert, P. M., Idler, W. W., Cabral, F., Gottesman, M. M. & Goldman, R. D. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3692–3696.
20. Steinert, P. M., Idler, W. W., Poirier, M. C., Katoh, Y., Stoner, G. A. & Yuspa, S. H. (1979) *Biochim. Biophys. Acta* **576**, 11–21.
21. Steinert, P. M., Idler, W. W. & Zimmerman, S. B. (1976) *J. Mol. Biol.* **108**, 547–567.
22. Steinert, P. M., Dyer, P. Y. & Rogers, G. E. (1971) *J. Invest. Dermatol.* **56**, 49–54.
23. Steinert, P. M., Peck, G. L., DiPasquale, A. & McGuire, J. S. (1976) *J. Invest. Dermatol.* **66**, 276.
24. Shelanski, M., Gaskin, F. & Cantor, C. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 765–768.
25. Steinert, P. M. & Idler, W. W. (1979) *Biochemistry* **18**, 5664–5669.
26. Teller, D. C. (1973) *Methods Enzymol.* **27**, 346–441.
27. Lee, J. C. & Timasheff, S. N. (1974) *Arch. Biochem. Biophys.* **165**, 268–273.
28. Renart, J., Reiser, J. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3116–3120.
29. Zackroff, R. V. & Goldman, R. D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6226–6230.
30. Balmain, A., Loehren, D., Fischer, J. & Alonso, A. (1977) *Dev. Biol.* **60**, 442–452.