# Protein complexes of intermediate-sized filaments: Melting of cytokeratin complexes in urea reveals different polypeptide separation characteristics

(cytoskeleton/keratin/vimentin/desmin/protein denaturation)

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ABSTRACT    Subunit complexes of cytokeratin polypeptides from intermediate-sized filaments (IF) of various tissues and cultured cells from rat, cow, and man were solubilized in low-salt buffer containing 4 M urea and exposed to increasing concentrations of urea, followed by urea gradient electrophoresis or two-dimensional gel electrophoresis at different urea concentrations. Correspondingly, cytokeratin polypeptides dissociated in 9.5 or 10 M urea were dialyzed into lower concentrations of urea and allowed to reassociate into specific complexes. It was found that the polypeptide constituents of a given cytokeratin complex dissociate in the form of a rather sharp "melting curve" and that dissociated polypeptides reassociate in the same mode of dependence on urea concentration. The midpoint of melting in urea $(U_m)$ is a characteristic property of a given complex of cytokeratin polypeptides. $U_m$ values differ markedly between different cytokeratin complexes, ranging from 5.9 to 9.0 M urea. The results also show that cytokeratins do not form complexes with vimentin, another type of IF protein. The data suggest that certain cytokeratin polypeptides are complementary and contain sequences that direct their association into specific complexes forming IF subunits.

In most vertebrate cells, a considerable portion of the total cellular protein is represented by cytoskeletal filaments of 7–11 nm in diameter—i.e., intermediate-sized filaments (IF), which contain proteins characterized by extended $\alpha$-helical regions (see refs. 1–3). Different IF proteins are expressed in different cell types so that specific categories of IF can be related to certain programs of cell differentiation (2, 4–6). Of the five major types of IF proteins (cytokeratins, vimentin, desmin, and glial filament and neurofilament proteins), the family of keratin-type proteins (cytokeratins), which is characteristic of epithelial cells (7–10), is very complex. Different polypeptides of this family can be expressed in different epithelia, and one cell type can contain a variable number of these polypeptides (6, 11–16).

Little is known about the arrangements of these polypeptides in IF and their protofilament subunits. In the course of electrophoretic studies of IF proteins, we have noted that certain cytokeratin polypeptides remain in complexes even at high concentrations of urea (17, 18). In the present study, we describe such complexes and show their dissociation by urea as well as their reassembly.

## MATERIALS AND METHODS

**Cells and Tissues.** Cultured cells of established lines (rat $MH_1C_1$; bovine MDBK and BMGE+H; human MCF-7, HeLa, and liver carcinoma SK-HEP1) were grown as described (13,

16–19) and, in some experiments, were labeled with L-[$^{35}$S]methionine (19). Sampling of tissues has also been described (13, 16).

**Cytoskeletal Preparations.** Cytoskeletal material from tissue samples was prepared as described (13, 16). Cell cultures were lysed and extracted with buffers containing Triton X-100 and high-salt concentrations (13, 19) and, in addition, 5 mM EDTA. To reduce the content of residual DNA, cytoskeletal pellets were often digested with *Staphylococcus aureus* nuclease (400 units/mg of protein) in 140 mM NaCl/10 mM Tris·HCl, pH 8.0/5 mM $CaCl_2$ for 1–2 min at 18–20°C. Digestion was stopped by the addition of EDTA to a final concentration of 5 mM. The cytoskeletal material was then washed with 140 mM NaCl/5 mM Tris·HCl, pH 8.0/5 mM EDTA. In some experiments, cytoskeletal proteins were labeled *in vitro* by $^{125}$I-radioiodination (20) or by reduction methylation with [$^3$H]NaBH$_4$ (21, 22).

**Gel Electrophoresis.** Cytoskeletal proteins were solubilized in 4 M urea/10 mM Tris·HCl, pH 8.0/25 mM 2-mercaptoethanol by stirring for 3 hr at room temperature. Small aliquots of supernatants obtained after centrifugation at 10,000 × g for 30 min were dialyzed for 3 hr against 1 mM Tris·HCl at pH 8.0 containing 25 mM 2-mercaptoethanol and various concentrations of urea. Alternatively, cytoskeletal proteins were solubilized in the same buffer containing 9.5 or 10 M urea, and the supernatant solutions were dialyzed against various lower concentrations of urea. For two-dimensional gel electrophoresis, the procedures of O'Farrell *et al.* (23, 24) were used with some minor modifications (13). Urea gradient gel electrophoresis was performed according to Creighton (25), mostly using gradients of 4–9.5 M urea and 6–10% acrylamide. Linearity of the urea gradients and absolute urea concentrations were determined by the inclusion of [$^{14}$C]urea. Gels were stained with Coomassie blue or by the silver staining procedure. In the case of radioactive samples, areas containing cytokeratin polypeptides were dissected from the gels and radioactivity per gel slice was determined.

## RESULTS

The simplest cytokeratin composition has been reported, in diverse species, for hepatocytes and various cultured hepatoma and kidney cells (6, 11, 13, 16, 19, 26). Cytokeratin filaments of these cells contain two polypeptides (A and D) corresponding to nos. 8 and 18 of the human cytokeratin catalog (16). We have solubilized cytokeratin from cytoskeletons of liver tissue and cultured cells ($MH_1C_1$ and MDBK) from rat and cow in solutions of 4 M urea, with recoveries of >70%. These complexes

Abbreviations: IF, intermediate-sized filament(s); IEP, isoelectric pH value revealed by two-dimensional gel electrophoresis; IEF, isoelectric focusing.

appear, on electron microscopy utilizing negative staining and rotary-shadow-cast techniques, as rods of ≈2 nm in diameter and ≈50 nm in length (data not shown) and contain polypeptides A and D in a 1:1 ratio, as judged from staining intensity with Coomassie blue. When this solubilized cytokeratin complex is examined by urea gradient gel electrophoresis (Fig. 1a), it migrates as a single band over the range from 4 to 5.5 M urea but splits into two bands, one containing polypeptide A and the other containing polypeptide D, when exposed to higher urea concentrations (Fig. 1 *a* and *b*). This method allows a direct visualization of the dissociation of IF polypeptides as a function of urea concentration.

Separation of the two polypeptides can also be studied by two-dimensional gel electrophoresis at different urea concentrations (Fig. 2a), taking advantage of the fact that the larger polypeptide A (in rat, $M_r$ 55,000) is less acidic [isoelectric pH value (IEP), 6.4] than polypeptide D ($M_r$ 49,000; IEP, 5.38). However, after isoelectric focusing (IEF) in 4 M urea, the two polypeptides are not separated but appear with the same IEP (Fig. 2b), indicating their inclusion in a complex. In solutions of urea concentrations >5 M, one observes a gradual separation of A and D, and in >7 M urea, the two polypeptides are completely separated (Fig. 2 *c–g*). Inversely, when cytokeratin solubilized in 4 M urea or total $MH_1C_1$ cytoskeleton is denatured in 9.5 M urea and then dialyzed against lower concentrations of urea, one finds that, at concentrations <7 M, an increasing proportion of D appears together with A (Fig. 2 *h–n*), and this process is complete at *ca.* 4.5 M urea. The midpoint ($U_m$) for both separation and reassociation is 5.9 M urea. A very
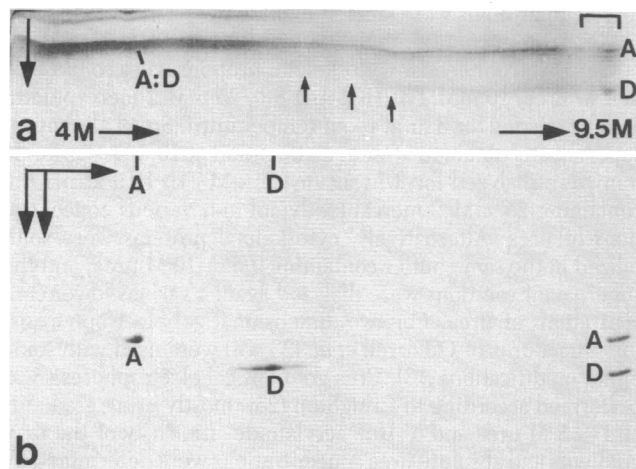


FIG. 1. Coomassie blue-stained gels showing urea gradient electrophoresis of cytokeratin complexes from rat $MH_1C_1$ cells solubilized in buffer containing 4 M urea. (a) Migration (downward arrow) in polyacrylamide (6–12%) gel with a 4 (left) to 9.5 M urea gradient perpendicular to the direction of electrophoresis (horizontal arrows) shows, in the left part, one band containing the complex of cytokeratin polypeptides A and D but reveals separation into a faster band (D) and a slower band (A) in regions of higher urea concentrations (upright arrows denote urea concentrations of 5.5, 6, and 7 M). The gel region denoted by the bracket on the right side was cut off and used for re-electrophoresis. (b) NaDodSO₄/polyacrylamide electrophoresis of polypeptides separated as described in *a*. A strip of the right (9.5 M urea) side of a gel similar to that shown in *a* was mounted (horizontal arrow) on top of a slab gel, equilibrated with sample buffer, and re-electrophoresed (downward pair of arrows). A and D on the top margin denote the original positions of these two polypeptides in the strip used; A and D in the gel denote the specific positions after electrophoresis; A and D in a reference lane of the same gel (right) denote these two polypeptides in co-electrophoresed cytoskeletal proteins. Electrophoresis in *b* proves that the two bands separated at higher urea concentrations in *a* represent dissociated polypeptides A and D.

similar dissociation and reassociation of polypeptides A and D is observed for the corresponding bovine complex (Fig. 3). In such experiments, another IF protein, vimentin, which is also present in MDBK cells (6, 26), is never associated with the cytokeratin polypeptides (Fig. 3). The human cytokeratins nos. 8 (A) and 18 (D) of SK-HEP1 cells also exhibit a similar melting and reassociation behavior (not shown).

The stability of cytokeratin complexes during melting in urea is not identical for all cytokeratins. Fig. 4 presents some examples of bovine cytokeratin complexes that are also soluble in 4 M urea but dissociate into their constituent polypeptides at urea concentrations higher than those required for A:D complexes. For example, the major cytokeratin polypeptides of bovine esophagus (13, 27, 29), the basic cytokeratin no. 6* ($M_r$ 59,000; IEP, 7.5) and the acidic cytokeratin no. 18 ($M_r$ 47,000; IEP, 5.25), form a complex focusing at an intermediate IEP of *ca.* 6.4, which is completely separated only at urea concentrations >7.5 M and reassociates into the same complex (Fig. 4 *b–e*) with a $U_m$ of 6.5 M urea (Fig. 5).

Epidermis of the same species displays a much higher complexity of cytokeratin composition of basic as well as acidic polypeptides (13, 27). When cytokeratin complexes from bovine muzzle epidermis are solubilized in 4 M urea, they display almost identical IEP values between pH 6.3 and 6.4 (Fig. 4f). At higher concentrations of urea, only one acidic component (designated VI) separates relatively early, and most of it is set free already at 6 M urea (e.g., Fig. 4g). Separation by melting of the other polypeptides takes place between 7 and 9.5 M urea (Figs. 4 *h–k* and 5). When bovine epidermal cytokeratins are dissolved in 10 M urea and dialyzed to various lower concentrations of urea, the basic components I, III, and IV form complexes with the acidic component VII (identical to no. 16; ref. 27) already at relatively high concentrations of urea, revealing the same curve as during melting (Fig. 5). By contrast, component VI is still separated at 6 M. Similar $U_m$ values have been found for the melting of polypeptides I and 16 in the mixture of total epidermal keratins or in material reconstituted from electrophoretically (13, 18) purified polypeptides I and 16 (not shown). Even more resistant to melting is the prominent cytokeratin complex of a basic cytokeratin of $M_r$ 59,000 (no. 6**; ref. 27) and an acidic cytokeratin of $M_r$ 50,000 (no. 16, identical to epidermal component VII) of cultured cells of the bovine mammary gland line BMGE+H (18). This complex requires as much as 10 M urea for complete separation (Fig. 4 *l–q*) and reveals a $U_m$ for both melting and reassociation of ≈9 M urea.

The IEP of a polypeptide can vary, to different extents for different proteins, with the concentration of urea (23). Therefore, we have examined various individual cytokeratin polypeptides that have been separated electrophoretically (13, 18) or by reversed-phase HPLC. With the exception of cytokeratin A (Figs. 2 and 3), the positions of diverse cytokeratin polypeptides at 4 M urea are different from those of their specific complexes (Fig. 4r). To check for exchange of cytokeratin polypeptides between different complexes in urea, we have mixed cytoskeletons from different cell types and incubated them in 4 M urea. The results (e.g., Fig. 4s) exclude exchange and reassociation under these conditions. We have also added purified individual cytokeratin polypeptides to native complexes in 4 M urea. The results (e.g., Fig. 4t) show that the cytokeratin polypeptides added do not co-focus with the complex, demonstrating that cytokeratin polypeptides do not associate with such complexes in an unspecific way.

When the separation of polypeptides of specific cytokeratin complexes is determined as a function of the concentration of urea, characteristic curves are obtained (Fig. 5). The melting profile of a specific cytokeratin complex is characterized by its
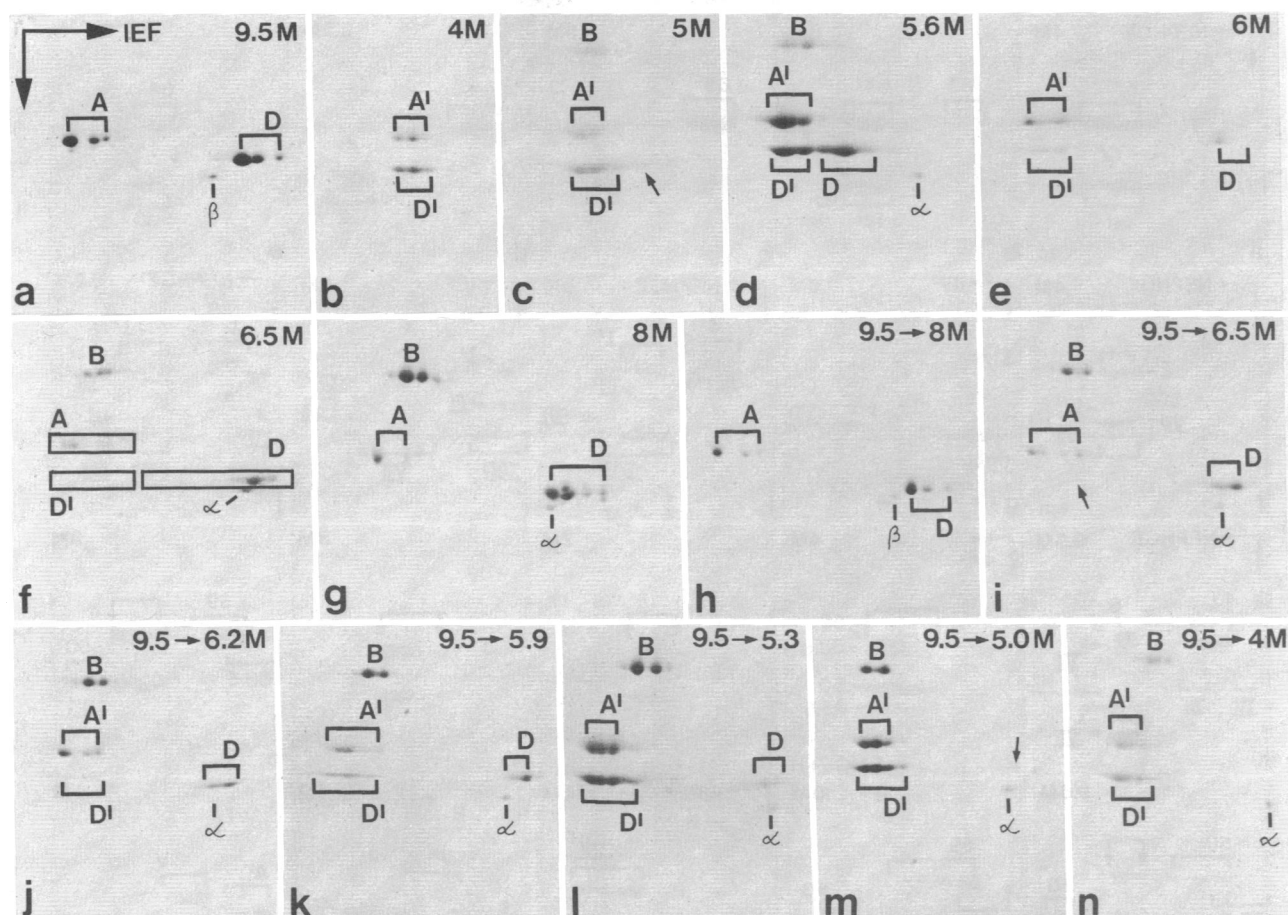
FIG. 2. Two-dimensional gel electrophoresis of cytokeratins from rat hepatoma MH₁C₁ cells (IEF in first dimension as indicated in *a*; downward arrow denotes direction of second-dimension electrophoresis in the presence of NaDodSO₄; acidic proteins are to the right). Urea concentrations and specific treatments are indicated in the upper right corners. B (bovine serum albumin) and $\alpha$ ($\alpha$-actin) denote co-electrophoresed reference proteins. (*a*) Total cytoskeletal proteins separated in 9.5 M urea, showing polypeptides A and D and residual $\beta$-actin ($\beta$). Note that D is much more acidic than A. (*b*) After isoelectric focusing in 4 M urea, A and D appear at positions A' and D'—i.e., with identical IEPs, due to their association in a complex. In this special case the denatured and dissociated form of A is not markedly different in IEP from that of A' in the A:D complex. (*c*) Dialysis and focusing in 5 M urea show a similar situation but also displacement of some polypeptide D (arrow). (*d*) On dialysis and focusing in 5.6 M urea, more of polypeptide D is displaced from position D'. (*e*) In 6 M urea, half of polypeptide D is displaced from position D' and recovered in position D. (*f*) In 6.5 M urea, one notes further separation of D from the complex-bound form D' (here the mode of excision of A, D', and D for determination of radioactivity is indicated by rectangles). (*g*) In 8 M urea, A and D are completely separated. (*h*) When cytokeratins are first denatured in 9.5 M urea, then dialyzed against buffer containing 8 M urea, and next focused in 8 M urea (denoted by "9.5 → 8M"), polypeptides A and D are still separated. (*i*) Similar to *h*, but after dialysis and focusing in 6.5 M urea, trace amounts of D are detected in the position of the complex (arrow). (*j*) Similar to *h*, but more cytokeratin D is in the position of the complex (D'). (*k*) Similar to *h*, but in 5.9 M urea, nearly equal amounts of D appear in the position of the free (D) and the complex-bound (D') polypeptide. (*l*) Similar to *h*, but most of D is recovered in position D'. (*m*) In 5 M urea, the shift of D to the complex position D' is nearly complete (some residual material in position D is denoted by arrow). (*n*) In 4 M urea, all cytokeratins co-focuses and the complex formed is indistinguishable from that directly solubilized in 4 M urea (*b*).
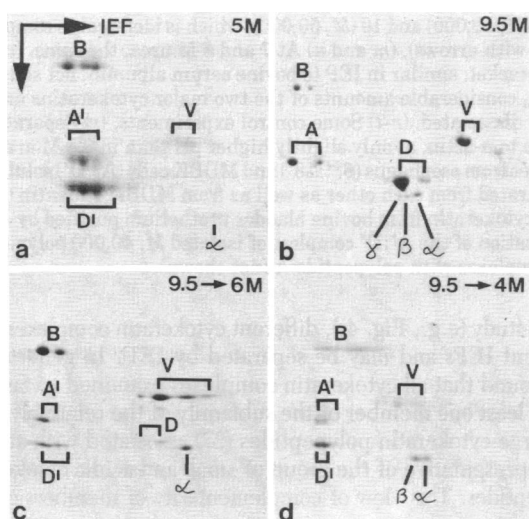
$U_m$ and sharpness, and different cytokeratin complexes can have drastically different $U_m$ values, ranging from 5.9 to 9.0 M urea.

## DISCUSSION

Subunit complexes of IF can be solubilized in low-salt buffers containing 4 M urea—i.e., conditions that for most proteins do



FIG. 3. Two-dimensional gel electrophoresis of the cytokeratin complex containing polypeptides A and D of bovine MDBK cells after solubilization in 4 M urea and subsequent dialysis and IEF in solutions of various urea concentrations (only some examples are shown; designations as in Fig. 2). (*a*) Both cytokeratins in complex position (A' and D'). (*b*) At 9.5 M urea, A and D focus separately ($\gamma$ denotes residual $\gamma$-actin). (*c* and *d*) When MDBK cytoskeletal material is solubilized in 9.5 M urea and then exposed to lower concentrations of urea, partial reformation of the complex (A' and D') is seen at 6 M urea (*c*) and complete recovery of D in position D' at 4 M urea (*d*). Vimentin (V), which when analyzed alone (not shown) shows an IEP shift from pH 5.3 (in 9.5 M urea) to *ca.* 5.9 (in 4 M urea; see *d*), is always separated from the cytokeratins.
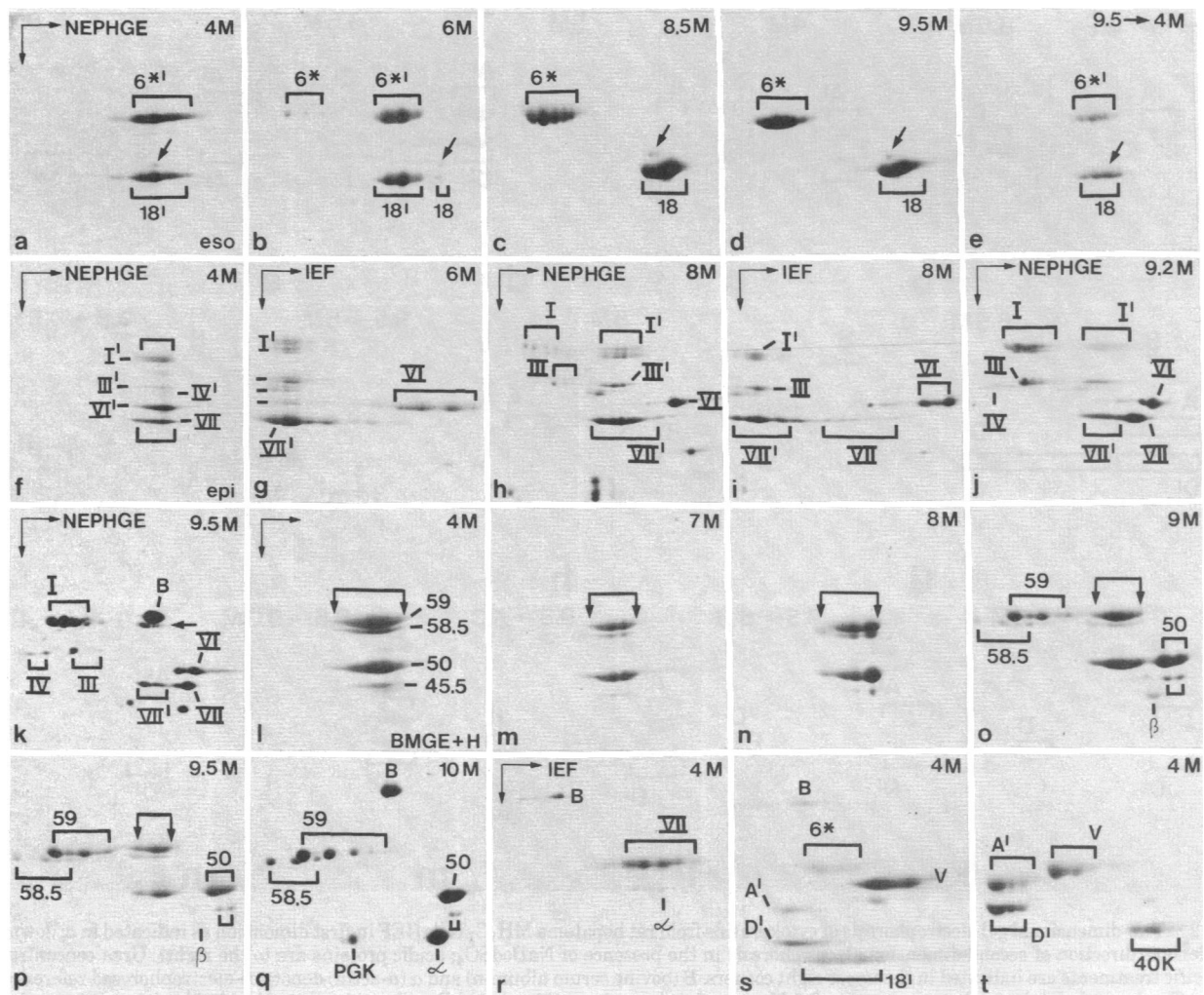
FIG. 4.    Two-dimensional gel electrophoresis [first dimension as nonequilibrium pH gradient electrophoresis (NEPHGE) or as IEF as in Fig. 2] of some bovine cytokeratin complexes containing basic polypeptides. (*a–e*) Cytokeratin from esophagus ("eso" in *a*) containing two major polypeptides designated 6* and 18 (arrow denotes a minor polypeptide). (*a*) At 4 M urea, both polypeptides comigrate in complex position (6*' and 18'). (*b*) At 6 M urea, only small amounts have left the complex and moved to the more basic (6*) or more acidic (18) position. (*c* and *d*) Complete separation of the two polypeptides at 8.5 (*c*) and 9.5 M (*d*) urea. (*e*) After denaturation in 9.5 M urea, followed by dialysis and electrophoresis in 4 M urea, the complex has formed again, focusing in the same position as in *a*. (*f–k*) Cytokeratin from bovine muzzle epidermis ("epi" in *f*) displaying several cytokeratin polypeptides (designated by roman numerals). (*f*) At 4 M urea, all polypeptides appear in the same position (brackets). (*g*) At 6 M urea, a large portion of component VI has dissociated, whereas the other polypeptides are still in the intermediate complex position, which is close to that of co-electrophoresed bovine serum albumin (not shown). (*h*) At 8 M urea, most of polypeptides I, III, IV, and VII are still in the intermediate complex position (bracket) but some have already become free and shifted. Component VI is completely free. (*i*) IEF separation of the same sample as shown in *h*. (*j*) At 9.2 M urea, most of the keratin polypeptides are free but some are still seen in complex position (bracket). Some material of the minor keratins III and IV is also still in complex position. (*k*) Even at 9.5 M urea, small amounts of polypeptides I (nos. 1–3; arrow) and VII (no. 16; bracket) are still in the position characteristic of the complex. (*l–q*) Separations (all NEPHGE) of cytokeratins from BMGE + H cells (arabic numerals indicate $M_r$ in $10^3$ units according to ref. 18); the two major components are polypeptides 6** ($M_r$ 59,000) and 16 ($M_r$ 50,000), which is identical to component VII of epidermis. (*l*) At 4 M urea, all cytokeratins are in complex position (bracket with arrows). (*m* and *n*) At 7 and 8 M urea, the same. (*o*) At 9 M urea, about half of each of the two has moved from the position of the complex (bracket; similar in IEP to bovine serum albumin, not shown) to the more basic and acidic positions of the free polypeptides. (*p*) Even at 9.5 M urea, considerable amounts of the two major cytokeratins are still in complex position (bracket). (*q*) Only at 10 M urea are the complexes completely dissociated. (*r–t*) Some control experiments. (*r*) Separation of isolated and purified bovine cytokeratin VII by IEF in 4 M urea. Note position close to α-actin, at only slightly higher pH than in 9.5 M urea (see *k, p,* and *q*). (*s*) Separation (IEF) of a mixture of equal amounts of cytokeratin complexes from esophagus (6*':18') and MDBK cells (A':D') solubilized in 4 M urea and incubated together. Note that both cytokeratin complexes are separated from each other as well as from MDBK vimentin (V). (*t*) Separation (IEF) of a mixture of cytoskeletal proteins from MDBK with $M_r$ 40,000 cytokeratin from bovine bladder urothelium purified by HPLC (designated 40K; see ref. 27) that had been incubated together for 12 hr. Note separation of the A':D' complex, of isolated $M_r$ 40,000 polypeptide, and of vimentin (V). In bladder, most of the $M_r$ 40,000 polypeptide is present in complexes with polypeptide A (not shown).

not result in extensive polypeptide chain unfolding (25, 29). Cytokeratin complexes prepared in this way appear as rod-like particles that migrate as a single band in electrophoresis and focus at an IEP characteristic of the specific complex. Despite the large differences in IEP of the individual cytokeratins, ranging from pH 5 to 8, cytokeratin complexes fall into a rather narrow range of IEP values (*ca.* 6.0–6.7). Nevertheless, as shown

in this study (e.g., Fig. 4*s*), different cytokeratin complexes have different IEPs and may be separated by IEF. In general, we have found that all cytokeratin complexes examined so far contain at least one member of the subfamily of the relatively basic and large cytokeratin polypeptides (27) associated with at least one representative of the group of small and acidic cytokeratin polypeptides. This view of complementarity of members of two
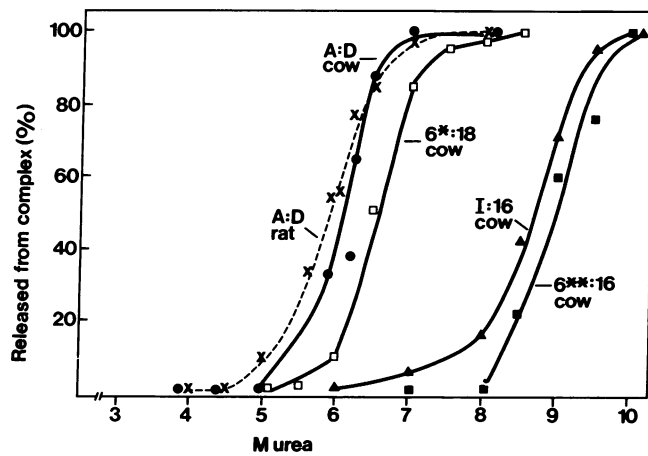
FIG. 5. A selection of melting curves of different cytokeratin complexes: ×, complex of A and D from rat hepatoma $MH_1C_1$ cells; ●, complex of A and D from bovine MDBK cells; □, complex of bovine cytokeratins nos. 6* and 18 of esophagus; ▲, complex of cytokeratins I (nos. 1–3) and 16 in bovine muzzle epidermis; ■, complex of bovine cytokeratins nos. 6** and 16 of BMGE+H cells. Values are from determinations of radioactivity; no substantial differences were found when the different labeling procedures were compared.

subfamilies in complex formation is also in agreement with IF reconstitution experiments (30) and nucleic acid hybridization data (31). We suggest that these complexes containing different cytokeratin chains are arranged in a coiled-coil α-helical structure and represent the actual subunits of protofilaments and IF.

With increasing concentrations of a denaturing agent, urea, the constituent chains of these cytokeratin complexes separate over a relatively narrow range of concentrations. The "melting curves" obtained allow the definition of a midpoint of melting ($U_m$), characteristic of the specific cytokeratin complex, at which equal amounts of the constituent polypeptides are free and complex-bound. Different cytokeratin complexes display differences in their polypeptide chain separation characteristics, and $U_m$ values can range from 5.9 M urea (for the A:D complex of liver and hepatoma cells) to 9.0 M (for the 6**:16 complex of BMGE+H cells). Curves obtained for the reassociation of denatured polypeptides into a specific cytokeratin complex are essentially identical to the melting curves. Certain cytokeratin polypeptides reassociate already in 8–9.5 M urea, suggesting that the domains involved in recognition and association of these chains are properly arranged at such high concentrations of urea (see refs. 29 and 32). It will be important to identify and localize the amino acid sequences that determine the intermolecular interactions resulting in the formation of these specific, complementary complexes.

The differences in melting between different cytokeratin complexes may reflect differences in the strengths of coiled-coil interactions that are related to the biological functions of these IF in the specific cells. In this respect, cell type specificity seems to be more important than species diversity. For example, complexes of cytokeratin polypeptides A and D from diverse species (man, cow, and rat) all have similar low $U_m$ values (5.9–6.2 M urea), whereas several epidermal keratin complexes from different species are much more resistant to melting.

Analyses of IF protein complexes and their melting are also

valuable in identifying topological relationships. For example, one cytokeratin polypeptide of bovine epidermis (no. VI) separates from all of the others at relatively low urea concentrations. Such analyses have also led to the detection of different cytokeratin complexes in the same cell (data not shown). We hope that the methods described here will also help in elucidating how the numerous polypeptides of this multigene family are combined with each other and how the assembly of these complexes is regulated.

1. Geisler, N. & Weber, K. (1982) *EMBO J.* 1, 1649–1656.
2. Lazarides, E. (1982) *Annu. Rev. Biochem.* 51, 219–250.
3. Steinert, P. M., Rice, R. H., Roop, D. R., Trus, B. L. & Steven, A. C. (1983) *Nature (London)* 302, 794–800.
4. Bennett, G. S., Fellini, S. A., Croop, J. M., Otto, J. J., Bryan, J. & Holtzer, H. (1978) *Proc. Natl. Acad. Sci. USA* 75, 4364–4368.
5. Franke, W. W., Schmid, E., Osborn, M. & Weber, K. (1978) *Proc. Natl. Acad. Sci. USA* 75, 5034–5038.
6. Franke, W. W., Schmid, E., Schiller, D. L., Winter, S., Jarasch, E.-D., Moll, R., Denk, H., Jackson, B. & Illmensee, K. (1982) *Cold Spring Harbor Symp. Quant. Biol.* 46, 431–453.
7. Schmid, E., Tapscott, S., Bennett, G. S., Croop, J., Fellini, S. A., Holtzer, H. & Franke, W. W. (1979) *Differentiation* 15, 27–40.
8. Sun, T.-T., Shih, C. H. & Green, H. (1979) *Proc. Natl. Acad. Sci. USA* 76, 2813–2817.
9. Franke, W. W., Weber, K., Osborn, M., Schmid, E. & Freudenstein, C. (1978) *Exp. Cell Res.* 116, 429–445.
10. Sun, T.-T. & Green, H. (1978) *Cell* 14, 469–476.
11. Franke, W. W., Denk, H., Kalt, R. & Schmid, E. (1981) *Exp. Cell Res.* 131, 299–318.
12. Franke, W. W., Winter, S., Grund, C., Schmid, E., Schiller, D. L. & Jarasch, E.-D. (1981) *J. Cell Biol.* 90, 116–127.
13. Franke, W. W., Schiller, D. L., Moll, R., Winter, S., Schmid, E., Engelbrecht, I., Denk, H., Krepler, R. & Platzer, B. (1981) *J. Mol. Biol.* 153, 933–959.
14. Fuchs, E. & Green, H. (1981) *Cell* 25, 617–625.
15. Wu, Y.-J. & Rheinwald, J. G. (1981) *Cell* 25, 627–635.
16. Moll, R., Franke, W. W., Schiller, D. L., Geiger, B. & Krepler, R. (1982) *Cell* 31, 11–24.
17. Moll, R., Franke, W. W., Volc-Platzer, B. & Krepler, R. (1982) *J. Cell Biol.* 95, 285–295.
18. Schmid, E., Schiller, D. L., Grund, C., Stadler, J. & Franke, W. W. (1983) *J. Cell Biol.* 96, 37–50.
19. Franke, W. W., Mayer, D., Schmid, E., Denk, H. & Borenfreund, E. (1981) *Exp. Cell Res.* 134, 345–365.
20. Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* 194, 495–496.
21. Means, G. & Feeney, R. (1968) *Biochemistry* 7, 2192–2201.
22. Heacock, C. S., Bernstein, B. W., Duhaiman, A. S., Amorese, D. A. & Bamburg, J. R. (1982) *J. Cell. Biochem.* 19, 77–91.
23. O'Farrell, P. H. (1975) *J. Biol. Chem.* 250, 4007–4021.
24. O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. (1977) *Cell* 12, 1133–1142.
25. Creighton, T. E. (1979) *J. Mol. Biol.* 129, 235–264.
26. Franke, W. W., Schmid, E., Grund, C. & Geiger, B. (1982) *Cell* 30, 103–113.
27. Schiller, D. L., Franke, W. W. & Geiger, B. (1982) *EMBO J.* 1, 761–769.
28. Milstone, L. M. (1981) *J. Cell Biol.* 88, 317–322.
29. Pace, C. N. (1975) *Crit. Rev. Biochem.* 3, 1–43.
30. Steinert, P. M., Idler, W. W. & Zimmerman, S. B. (1976) *J. Mol. Biol.* 108, 547–567.
31. Fuchs, E. V., Coppock, S. M., Green, H. & Cleveland, D. W. (1981) *Cell* 27, 75–84.
32. Privalov, P. L. (1982) *Adv. Protein Chem.* 35, 1–104.