Conservation of the Structure of Keratin Intermediate Filaments: Molecular Mechanism by Which Different Keratin Molecules Integrate into Preexisting Keratin Intermediate Filaments during Differentiation

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ABSTRACT: During development and differentiation, the intermediate filament component of the cytoskeleton of many cells and tissues is rebuilt by a dynamic exchange process in which one set of protein chains is replaced by another, without recourse to creation of a new network. One major example is the replacement of keratin 5/keratin 14 (K5/K14) keratin intermediate filaments (KIFs) by K1/K10 KIFs during terminal differentiation in the epidermis. The present work was undertaken to explore how this may occur. We have induced lysine–lysine cross-links with disulfosuccinimidyl tartrate in K5/K14 KIFs in order to determine the axial dimensions and relative axial alignments of the K5/K14 molecules. Many of the cross-links induced in subfilamentous oligomers containing one, two, or three molecules were also found in the intact KIF, indicating that the body of data thus generated provides physiologically relevant information on the structural organization in the KIF. A least-squares analysis using as data the positions of lysine residues involved in 23 induced cross-links has allowed the axial alignments of the various coiled-coil segments in the rod domain to be determined. Three modes of antiparallel alignment of two neighboring molecules were found: A_{11} (staggered by −16.7 nm), A_{22} (staggered by 28.8 nm), and A_{12} (almost in register; staggered by only 0.3 nm). Since the axial repeat length is about 1 nm less than the molecular length, the data require a fourth mode of molecule alignment, termed A_{CN}, in which similarly directed molecules are overlapped by the equivalent of about 5–10 residues. Interestingly, these axial alignments and dimensions are essentially identical to those adduced previously for K1/K10 KIF [Steinert, P. M., Marekov, L. N., Fraser, R. D. B., & Parry, D. A. D. (1993) *J. Mol. Biol. 230*, 436–452], thus indicating that the two types of KIF have conserved structures. Accordingly, our new data suggest that exchange of the K5/K14 molecules by K1/K10 molecules can occur simply because both have the same linear dimensions and axial configurations. Further work will be necessary to determine whether the lack of assembly compatibility of molecules in other IF systems is due to variations in their axial dimensions and alignments.

A large family of keratin chains constitute the intermediate filament (KIF)[1] component of the cytoskeleton of epithelial cells (Steinert & Roop, 1988; Goldman & Steinert, 1990; Albers & Fuchs, 1992). KIFs assembled from denatured protein chains in vitro (Steinert et al., 1976; Hatzfeld & Franke, 1985) and KIFs of epithelial cells in vivo (Sun et al., 1984) are obligate heteropolymers, consisting of one type I and one type II chain (Parry et al., 1985; Hatzfeld & Weber, 1990; Steinert, 1990). Most epithelial cell types express a particular pair of chains, often characteristic of the epithelium (Sun et al., 1984). For example, basal cells of stratified squamous epithelia, including the epidermis, express the keratin 5 and keratin 14 (K5/K14) pair of chains; epithelia which undergo terminal differentiation, such as the suprabasal cells of the epidermis, down-regulate this pair and begin to express the K1/K10 pair, which eventually constitute the bulk of the tissue.

Recent studies have documented certain aspects of the hierarchical structure of KIFs. A type I/type II heterodimer coiled-coil molecule serves as the fundamental unit (Hatzfeld & Weber, 1990; Steinert, 1990). Under conditions in vitro in which the total protein concentration (C_o) is less than the critical concentration (C_c), assembly proceeds only as far as a two to four molecule sized oligomer (Steinert, 1991a). Recent cross-linking experiments with human and mouse K1/K10 oligomers (Steinert & Parry, 1993; Steinert et al., 1993) have established that there are four modes of association of two neighboring molecules with respect to each other and have defined their exact axial parameters: A_{11}, in which two molecules are antiparallel and staggered by 16 nm so as to overlap their 1B coiled-coil rod domain segments; A_{22}, antiparallel and staggered by 28 nm, in which the rod domain 2 segments overlap; A_{12}, in which two antiparallel molecules are aligned almost exactly in register; and A_{CN}, deduced from A_{11} and A_{22}, in which the last 1.6-nm section (corresponding to about 10 residues) of the 2B rod domain segment of one molecule overlaps the first 10 residues of the 1A rod domain segment of its similarly directed neighbor. Thus, a two-dimensional surface lattice for the K1/K10 KIF consists of about 16 molecules arranged in alternating in-register and staggered antiparallel rows (Steinert, 1991a,b; Steinert et al., 1993). These modes of alignment had been predicted earlier in all types of IF on the basis of several types of indirect data

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[1] Abbreviations: C_o, total protein concentration; C_c, critical concentration; DST, disulfosuccinimidyl tartrate; DTT, dithiothreitol; HPLC, high-performanace liquid chromatography; IF, intermediate filaments; KIF, keratin intermediate filaments; K, keratin, so that K5 = keratin 5 chain.

and confirm several previously identified axial periodicities (Crewther et al., 1983; Fraser et al., 1986, 1990). However, it is not yet known how this lattice folds in three dimensions into a KIF about 10 nm in diameter (Fraser et al., 1990). Nevertheless, it is clear that a prominent discontinuity in the regularity of molecular packing will occur, leading to a seam spiraling along the axis of the KIF (Fraser & MacRae, 1983; Steinert et al., 1993). We have predicted that molecules can exchange along this seam, leading to local variations in packing density and KIF structure (Steven et al., 1983; Steven, 1990; Steinert et al., 1993). However, some uncertainty exists as to the general applicability of the K1/K10 KIF model for all other IFs. On the basis of detailed examinations of the axial parameters revealed by low-angle X-ray diffraction data, the exact organization of molecules in invertebrate neurofilaments (Day & Gilbert, 1972) and wool keratin KIF (Fraser & MacRae, 1983) were found to be different (Fraser et al., 1990). While limited cross-linking studies suggest that type III desmin IFs contain an A_{11}-like molecular alignment (Geisler et al., 1992), another study (Coulombe & Fuchs, 1990) has reported that K5/K14 KIFs are built from molecules aligned only in an A_{12} -like mode.

In this paper we show that the organization of molecules in K5/K14 KIF is indeed identical to that of K1/K10 KIF. Combination of the two sets of data has permitted a refinement of the fundamental axial parameters of epidermal KIF that should also aid in the construction of working three-dimensional models. Furthermore, the data afford a simple mechanism by which KIFs undergo dynamic exchange in cells, which also has important implications for diseases involving the keratins.

MATERIALS AND METHODS

Isolation of Proteins. The epidermis of freshly-excised human foreskins was separated by overnight flotation on trypsin, recovered, dispersed into individual cells, and plated for 4 h essentially as described (Marrs & Vorhees, 1971; Steinert & Yuspa, 1978). Those cells which attached in this time consisted mostly of basal cells (Yuspa & Harris, 1974). The cells were recovered, and cytoskeletons were prepared (Steinert et al., 1982), which were then extracted in a buffer of 8 M urea, 50 mM Tris-HCl (pH 8.0), 1 mM DTT, and 1 mM EDTA and clarified by centrifugation at 110000*g*. Analysis by polyacrylamide gel electrophoresis confirmed that the extract contained the highly purified K5 and K14 chains as well as small amounts of actin and tubulin and certain very high molecular weight proteins. The K1 and K10 chains of the suprabasal keratinocytes were present in trace amounts only. The K5 and K14 chains were then isolated in pure form by preparative electrophoresis on 3-mm-thick slabs of a 7.5% polyacrylamide gel, excised, recovered by electroelution, and freed of SDS into the above urea buffer, and their concentration was determined spectrophotometrically (Steinert, 1990). In this way 5–7 mg each of K5 and K14 were recovered from the basal cells of 15 foreskins.

In vitro KIF reassembly was performed by dialysis of equimolar mixtures of the K5 and K14 chains (C_o of 0.05–1 mg/mL) into solutions of decreasing urea concentrations for 2 h each, from 6 to 4 to 2 to 1 to 0 M, in 5 mM Tris-HCl (pH 7.6), 1 mM DTT, and 1 mM EDTA (Steinert, 1990). The assembly competence of the mixture was assessed by a centrifugation assay (Steinert, 1990), electron microscopy following negative staining (Steinert, 1991b), and turbidity (see below) (Steinert, 1991a).

For cross-linking experiments with disulfosuccinimidyl tartrate (DST), the K5/K14 equimolar mixtures were dialyzed into 10 mM triethanolamine hydrochloride (pH 8.0) at either 0.05 or 0.5 mg/mL. In the former, below the critical concentration (C_c), assembly proceeds only as far as small oligomers (Steinert, 1991a; see Figure 1a) of one-, two-, three-, and traces of four-molecule species.

Cross-Linking Procedures. Cross-linking was performed with DST (Bragg & Hou, 1980) exactly as described previously (Steinert et al., 1993) and quenched with 0.1 M NH_4HCO_3. In initial experiments, the optimal conditions for cross-linking of KIF oligomers formed below the C_c for assembly were determined in a series of reactions with the amount of reagent varying between 0.1 and 2 mM. Other experiments were also performed to assess the extent of modification of lysine side chains on the keratins, in order to optimize the yield of cross-linked peptides following tryptic digestion. This was done by chemical reaction with 0.1 M iodoacetate at pH 9.5 and stopped after 30 min at 23 °C by addition of excess DTT. This quantitatively modifies the sulfhydryl and ϵ-NH_2 groups to their *S*-carboxymethyl and *N*-carboxymethyl derivatives, respectively (Waxdal et al., 1968). After dialysis to remove salts, amino acid analysis was then used to estimate the amount of unmodified lysine. The DST adduct is hydrolyzed in acid back to free lysine, but the *N*-carboxymethyl derivative is not and elutes away from the free lysine peak. Following a series of control experiments, it was determined that reaction with 0.4 mM reagent for 30 min recovered significant yields of one-, two-, and three-molecule oligomers with a minimum of chemical modification. Subsequent to reaction, cross-linked oligomers were recovered from solution by precipitation with 0.3 M sodium acetate (pH 5.0), pelleted, and redissolved in gel loading buffer containing 1% SDS, and the one-, two-, and three-molecule oligomers were resolved preparatively on a 3.0-mm-thick 3.75–17.5% gradient polyacrylamide slab gel (Steinert et al., 1993). The oligomers were finally freed of SDS and glycine salts, redissolved in the above urea buffer, and equilibrated in 0.1 M NH_4HCO_3.

Similar conditions were used for cross-linking of intact long K5/K14 KIFs, except that the protein concentration was higher (0.45–0.5 mg/mL). In this case, the products were recovered by precipitation at pH 5, resuspended in 70% formic acid (1 mg/mL), and cleaved with CNBr (1:1 by weight) for 18 h at 23 °C. During the reaction, the protein became soluble. This reaction mixture was diluted 10-fold in water and freeze-dried, and the peptides were redissolved in 0.1 M NH_4HCO_3.

Light-Scattering Assays. Turbidity was used to measure KIF assembly in vitro (Steinert et al., 1976; Steinert, 1991a). In this case, the urea was removed rapidly by passage through a Sephadex G-25 column (Steinert, 1990, 1991b). Samples (C_o = 0.50 mg/mL) were measured at 310 nm in a Beckman DU-65 spectrophotometer at 1-min intervals for 2 h at 37 °C. In assembly competence reactions, 5–20 μL of either equimolar mixtures of K5/K14 protein or one-molecule cross-linked oligomers at 12 mg/mL in 6 M urea was added at 10 min. This increased C_o to 0.6–0.95 mg/mL. Control reactions showed that KIF asembly is not inhibited in the presence of 0.1–0.2 M urea (Steinert, 1990).

Recovery and Analysis of Cross-Linked Peptides. The DST cross-linked products were digested to completion with trypsin (Sigma, bovine, sequencing grade) using 2% enzyme (w/w) for 6 h at 37 °C. A second 1% (w/w) amount of enzyme was added for a further 8–12 h of digestion. Tryptic peptides were then resolved by reverse-phase HPLC (Beckman 128/168 with System Gold Software) using a 60-min gradient of 0–40% acetonitrile in 0.1% trifluoroacetate. An aliquot of about 0.1 nmol of cross-linked products was run and compared

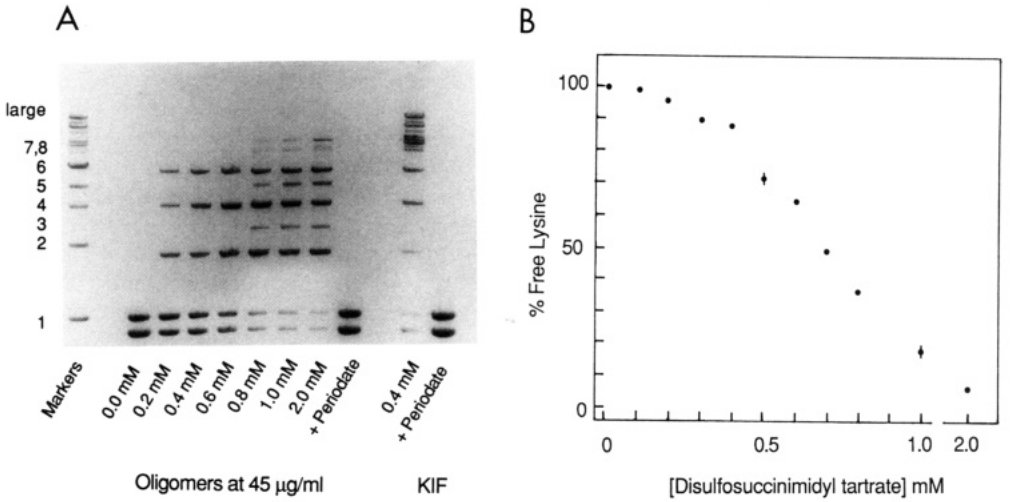

FIGURE 1: Cross-linking of human K5/K14 oligomers and KIFs by DST. (A) A 3.75–7.5% gradient polyacrylamide gel showing equal loads (1.1 μg) of cross-linked products of a solution of K5/K14 oligomers formed at C_o = 45 μg/mL (lanes 1–8) at different concentrations of DST or intact K5/K14 KIFs (lanes 9,10) at 0.4 mM DST, is shown. Densitometric scanning of the amount of monomer K5 and K14 chains was used to estimate the total amount of protein in oligomers. (B) Estimation of the degree of modification of lysine ε-NH_2 following reaction by DST with iodoacetate at pH 9.5 and then amino acid analysis. Together, these data show that as much as 50% of the protein can be cross-linked at 0.4 mM DST with as little as 10% lysine side-chain modification.

with a similar sample that had been cleaved with 0.1 M sodium periodate in 30 min. Alignment of the two profiles revealed several obvious peaks present in the former that were absent from the latter, thus representing potential cross-linked peptides. Such peptides were then recovered in a preparative run. Most potential cross-linked peptides were purified in a second round of HPLC fractionation. Finally, all cross-linked peptides were freeze-dried and redissolved in 0.1 M NH_4HCO_3. Each was then digested with 1% of the V8 protease (Sigma, type XVIIb) for 1 h at 37 °C and cleaved with periodate, and the products were resolved by HPLC, as described in detail previously (Steinert & Parry, 1993; Steinert et al., 1993). In most cases two well-resolved major peaks, and a few minor peaks resulting from incomplete V8 digestion, were clearly resolved away from the elution time position of the cross-linked peptide. Each of these shifted peptides, representing fragments of the "arms" of the cross-linked peptides, was recovered and dried. Each was then hydrolyzed in 5.7 N HCl at 108 °C in vacuo for 16 h and its amino acid composition was determined on a Beckman 6300 analyzer.

Determination of Axial Parameters by Least-Squares Fitting Methods. Each of the characterized cross-links was readily assigned by inspection to one of three modes of molecular assembly, in which pairs of molecules are arranged antiparallel to one another (see Figure 6). In the first, the molecules are largely overlapped (A_{12}); in the second, their 1B segments are overlapped (A_{11}); and in the third, their 2B segments are overlapped (A_{22}). The axial staggers between molecules in these three modes are all of the form Δz(U,D), where U and D refer to up- and down-pointing molecules, respectively (Fraser & MacRae, 1985; Fraser et al., 1986). Numerically Δz(U,D) can be calculated in terms of residue numbers i_1 and i_2 in the two molecules concerned, and can be shown to have the value ($i_1 + i_2 - 276 - L1 - L12 - L2 - 1$), where $L1$, $L12$, and $L2$ are the axially projected link lengths measured in multiples of h_{cc}, the average axial rise per residue in a coiled-coil conformation (Steinert et al., 1993). The number 276 is the total number of residues in the four coiled-coil domains 1A, 1B, 2A, and 2B, each of which has an axial rise of 0.1485 nm/residue. The equations thus generated for each of the unique cross-links identified were subjected to a least squares analysis in which the values of the six parameters $L1$, $L12$, $L2$, $\Delta z(U,D)_{12}$, $\Delta z(U,D)_{11}$, and $\Delta z(U,D)_{22}$ were refined (Steinert et al., 1993), from which the exact degrees of overlap for the three modes of alignment were calculated. In some cases (see text) certain of these parameters (and in particular $L12$) were imperfectly defined, as the number of equations which specify them was insufficient to give a meaningful value. In such instances one or more parameters were held constant and the others refined as usual.

RESULTS

Efficient Cross-Linking of Human K5/K14 KIFs and Oligomers with DST. Figure 1 A shows a polyacrylamide gel of a series of cross-linking reactions with the bifunctional cleavable reagent DST. At $C_o < C_c$, estimated from light scattering experiments to be < 50 μg/mL (data not shown; Steinert, 1991a), assembly of K5/K14 KIF proceeds only as far as the three-to-four-molecule stage, exactly as shown previously for K1/K10 KIF (Steinert, 1991a). This means that K5/K14 KIF assembly in vitro, and probably in vivo as well, is a rate-limited nucleation-dependent reaction. Also, as found for K1/K10 KIF, small amounts (<5% of protein) of K5/K5 and K14/K14 homodimer molecules formed in these in vitro assembly reactions.

Reaction with 1–2 mM of DST reagent leads to essentially complete cross-linking of the oligomers in solution, so that about 30% of the total protein consists of the one molecule species, about 50% is the two-molecule species, and 10% is the three-molecule species; smaller amounts (1–2%) of the four-molecule species are detectable (Figure 1a). Titration with iodoacetate was performed to assess the extent of the modification of the lysine ε-NH_2 groups (Figure 1B). On the basis of the published sequences of the human K5 (Eckert & Rorke, 1988; Lersch et al., 1989) and K14 chains (Marchuk et al., 1984), there are 53 lysine residues per K5/K14 heterodimer molecule of 110 kDa. Amino acid analyses revealed about 4.8 mol of unmodified lysines/110 kDa (that is, estimated 48.2 mol of DST adducts, including cross-links, per mol), indicating the reaction with 2 mM DST had achieved about 92% completion of modification. However, the presence of significant amounts of odd-numbered oligomers (Figure 1A) suggests that a troublesome degree of cross-linking of random-collision complexes occurs with 0.8–2 mM reagent.

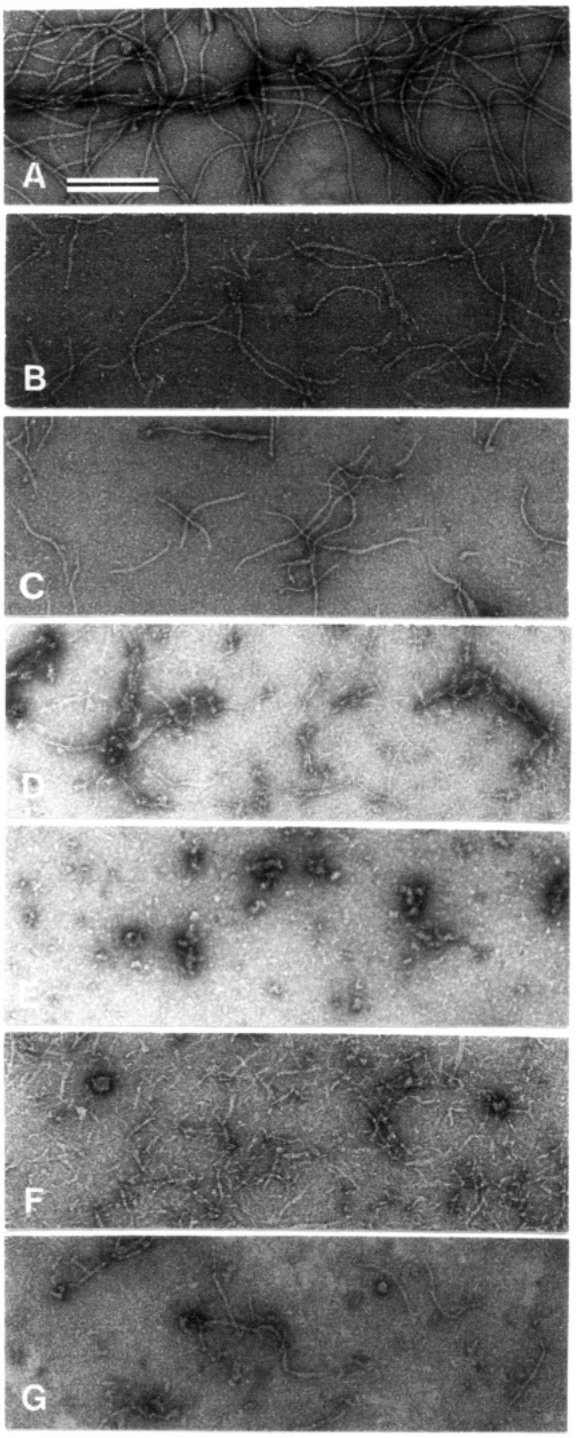

FIGURE 2: Assembly competence of cross-linked human K5/K14 oligomers as determined by electron microscopy. (A) Unreacted K5/K15 KIFs reassembled from a reconstituted equimolar mixture of chains. Following cross-linking at 0.4 mM DST, the one-molecule (B) and two-molecule (C) oligomers were recovered and reassembled in short but typically 10 nm wide KIFs. (D) The two-molecule oligomers recovered from cross-linking with 1 mM DST were largely assembly incompetent, due to excessive modification of lysine side chains. (E) Equimolar mixtures of normal K14 with SCM-K5 protein, in which only the sulfhydryl group had been modified by iodoacetic acid, were also assembly incompetent. In a second control experiment, intact KIFs were cross-linked with 0.4 mM DST and were either denatured and then reassembled (F) or cleaved with periodate, denatured, and then reassembled (G); in neither case was the protein assembly competent. Keratins were negatively stained with freshly filtered 0.7% uranyl acetate. Bar = 0.2 μm.

At 0.4–0.6 mM, where no odd-numbered oligomers were seen, the yield of cross-linked species appears to be about 40 and 50% completion, and titration data reveal 6.6 and 19.1 mol of adducts/mol (about 12 and 36% net of the total lysine side chains have been chemically modified) (Figure 1B). Thus reaction with 0.4 mM was chosen in this study since it affords a good yield of cross-linked oligomers with a minimum of protein modification. Using these conditions, intact KIFs were cross-linked to very large species (Figure 1A) with about 10% modification of lysines (5.2 mol of adducts/110 kDa). With both intact KIFs and KIF oligomers, the cross-links could be completely cleaved by treatment with 0.1 M sodium periodate in 30 min (Figure 1A).

KIF Oligomers Cross-Linked by DST Are Assembly Competent. By use of electron microscopy of negatively stained specimens, we found that the one-molecule (not shown), two-molecule (Figure 2B), and three-molecule oligomers (Figure 2C), containing about 6.6 mol of modified lysines/mol, formed typical KIFs in vitro, albeit somewhat shorter than those formed from recombined and unmodified K5 and K14 chains (Figure 2A). On the other hand, the one-molecule oligomer formed in a cross-linking reaction with 1 mM DST, containing about 43.2 mol of cross-links/mol, could no longer form KIF-like structures in vitro (Figure 2D). Similarly, mixtures of the unmodified K14 chain with the K5 chain in which the cysteines (two in the rod domain) had been converted to *S*-carboxymethyl derivatives by reaction with iodoacetate (Steinert & Parry, 1993) were no longer assembly competent (Figure 2E), suggesting that the cysteines are critical for normal KIF assembly. Two other control experiments were performed with cross-links induced in intact KIFs with 0.4 mM reagent: in one, the cross-linked KIFs were denatured in urea and reassembled; in the second, the cross-links in the KIFs were first cleaved with periodate and then reassembled. In the second test, the periodate should cleave all cross-links and remove the bulky adduct from those lysine side chains that were not in fact cross-linked. In neither case was the protein assembly competent (panels F and G, respectively, of Figure 2). Even though there were only about 6.5 mol of cross-links/mol, comparable to the degree of reaction with oligomers, we think the major reason for assembly failure is modification of certain key lysines that are essential for either KIF assembly or stabilization.

In a further test, we used light scattering to assess the assembly competence of the cross-linked one-molecule species (Figure 3). The rate of assembly of K5/K14 protein into KIFs in vitro follows a sigmoidal curve, exactly as shown previously for bovine (Steinert et al., 1976) or mouse KIF (Steinert, 1991a). In comparison to addition of buffer alone, addition of unmodified K5/K14 protein increased the rate of reaction, as expected. Addition of the one-molecule oligomer cross-linked by 0.4 mM DST (6.6 mol of adduct/mol) also increased the rate of reaction to the same extent, indicating that it could fully participate in normal KIF assembly. However, addition of one-molecule oligomer cross-linked with 1 mM DST (43.2 mol of adduct/mol) reduced the rate of reaction, suggesting that it could not participate in KIF assembly; indeed, the reduction in turbidity imples that it poisoned the reaction by interfering with KIF assembly and promoted some disassembly at the highest concentration added.

Together, these data indicate that the oligomers used in these cross-linking experiments are indeed assembly competent, providing the degree of protein modification is low. Therefore, analyses of the cross-links induced in them are likely to provide structurally relevant information about the alignment of their molecules.

Analysis of Cross-Links. The particular advantage of cross-linking with DST is that it has a short glycol cross-link arm

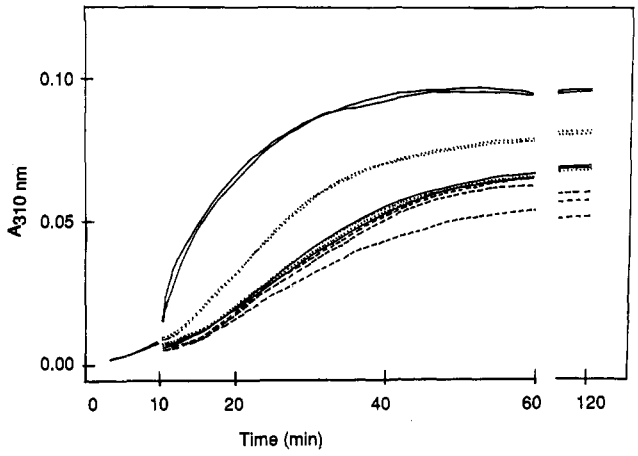

FIGURE 3: Use of turbidity to assess assembly competence of cross-linked one-molecule species. To a $C_o = 0.5$ mg/mL assembly reaction mixture were added 20 (solid lines) or 10 μl (dotted lines) of a 12 mg/mL K5/K14 protein mixture, one-molecule oligomer containing 6.6 mol/mol of DST adducts, and 6 M urea or H_2O were added at 10 min, and further turbidity changes as KIF assembly proceeded to completion were followed for 2 h. In a similar reaction, 5, 10, or 20 μl of one-molecule oligomer containing 48.2 mol/mol of DST adducts did not support further KIF assembly (broken lines).

(0.6 nm) which can be cleaved with 0.1 M sodium periodate (Bragg & Hou, 1980). Figure 4B-D shows fractionation profiles of tryptic peptides recovered from cross-linking reactions of the one-, two-, and three-molecule oligomers. The peaks which appeared as a result of the cross-linking reaction, and which disappeared upon cleavage with periodate (Figure 4E for three-molecule oligomers), are highlighted. In general, there was a shift of short polar peptides (generally <8 residues) eluting between 8 and 16 min that were unresolved toward a number of well-resolved peaks eluting at later times, corresponding to increases in mass due to cross-linking. The several new major peaks were reproducible between different experiments, but the height of the baseline of the profiles varied somewhat, apparently due to the varying presence of many minor cross-linked species (Steinert et al., 1993). A total of 45 major peaks were recovered, subjected to partial digestion with V8 protease, and cleaved with periodate, and the peptide arms were recovered for amino acid analysis. Most of these V8 peptides were quite short (<10 residues), and since they were recovered in high yield (generally >0.25 mol/mol) and were pure, the resulting analyses revealed simple compositions which in most cases permitted identification of the peptides along the K5 or K14 chains of known sequences. We found that the V8 digestion did not proceed to completion in most cases (examples of which are shown in Figure 5C–F). This afforded the opportunity to obtain additional likely sequence information in the vicinity of the cross-link. In this way, we identified the likely sequences of the two peptide arms of 39 peaks. In three other cases, sequences corresponding to only one peptide arm were recovered. We think these arose because a peptide was cross-linked to itself since the peptide was recovered in high yield (>0.2 mol/mol), a value which is too high to be explained by the known minor presence of homooligomers in these protein samples [estimated to be 5% of total protein (Figure 1A)]. Three minor peptide fractions could not be identified using these methods, as their amino acid compositions were not reproducible, and they may have been peptides resulting from cross-links arising from disulfide exchange or homooligomers. In total, 26 unique cross-linked peptides were identified (Table I).

Similarly, 20 cross-linked peptide fractions were recovered from the reaction with intact KIF, all of which were informative

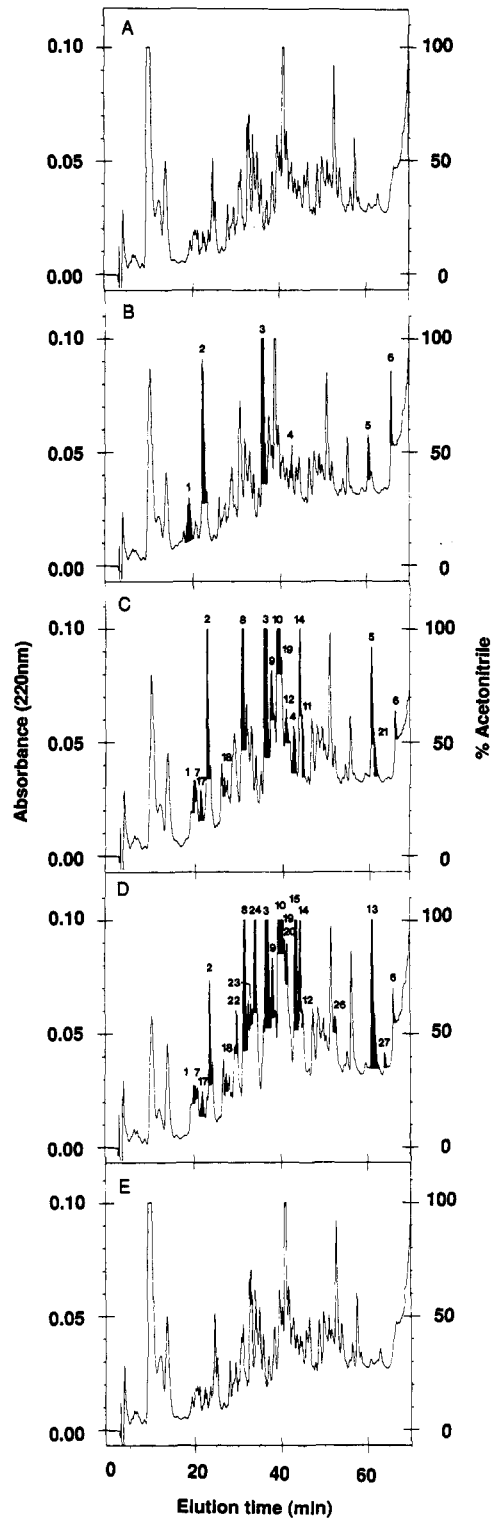

FIGURE 4: HPLC fractionation of cross-linked tryptic peptides of human K5/K14 oligomers. Peptide profiles are from (A) non-cross-linked KIF protein digested at 45 μg/mL, (B) cross-linked one-molecule oligomers, (C) cross-linked two-molecule oligomers, (D) cross-linked three-molecule oligomers, and (E) cross-linked three-molecule oligomers treated with periodate before fractionation. Comparisons of profiles A and E with profiles B-D revealed shifted peaks of cross-linked peptides (*shaded*) that were recovered for analysis.

(Table I). In this case, it was necessary to perform a CNBr digestion step in order to achieve subsequent complete proteolysis by trypsin (Figure 5). While this altered the HPLC profiles of fractionated peptides, significantly, 16 involved the same lysines as found above.

Table I lists all of the informative cross-links identified in Figures 4 and 5. Several of the 30 species were recovered repeatedly. Our calculations suggest that of a theoretical possible total of ten K5/K14 intramolecular cross-links, as expected from established structural principles (Hatzfeld & Weber, 1990; Steinert, 1990), six were routinely found (Figure 6). Twenty-three intermolecularly cross-linked peptides could be assigned to one of three alignments (Figure 6): the A_{11} and A_{22} modes suggest antiparallel staggered alignments at the two-molecule level, and the A_{12} mode, suggesting an antiparallel alignment in near-registration, occurred only at the three-molecule level, or in KIFs, in concurrence with our earlier data (Steinert, 1991a,b; Steinert et al., 1993). Only peptide 30 involving a cross-link between end domain sequences in KIF could not be unequivocally assigned. All but four cross-linked species involved lysines that occupy the *e*, *f*, or *g* heptad positions of the coiled-coil, as predicted by Parry (1990).

We have also noted that the same lysine residue was often cross-linked to multiple partners in different intra- or intermolecular alignments, among which the following occurred three or more times: K5, 1A-10; K5, 1B-89; K5, 2B-23; K5, 2B-83; K14, 1B-6; and K14, 1B-89/90. We are exploring the possibility that these are key residues in defining higher orders of KIF structure. Interestingly, mutations that change residue 1A-10 (an arginine) in the K14 or K10 chain are the proximal causes of at least two forms of pathology (Epstein, 1992; Fuchs, 1992).

Calculation of Rod Axial Alignments and Linker Segments in K5/K14 KIF. Using the 23 unique intermolecular cross-links, separate equations were derived (Table II) which relate directly to the positions of the lysine residues in the unique cross-links.

All of the equations can be expressed in terms of six parameters (A_{12}, A_{11}, A_{22}, $L1$, $L12$, and $L2$), which are refinable using conventional least-squares methods. In several cases, however, the equations are not independent. For example, eqs 11–16, 18, 19, and 20–23 in Table II are such that each of these groups effectively result in only a single constraint in the least-squares analysis. Nevertheless, certain axial staggers such as Δz(1BU,1BD), Δz(2BU,2BD), and Δz(1BU,2D) are very well defined by the cross-links and tally closely with those from the K1/K10 analysis (Steinert et al., 1993) (Table III). Indeed many of the cross-links involve lysines in the same positions reported for the K1/K10 oligomers (Steinert et al., 1993). Similarly, it is clear that A_{12}, L_1, and to a slightly lesser extent $L2$ are also well defined by the available data (Table III). These results confirm the A_{12}, A_{11}, and A_{22} modes of alignment reported for K1/K10 oligomers, the lengths of the linkers L_1 and L_2, and the relative axial staggers between antiparallel 1B–1B, 2B–2B, and 1B–2B segments.

The values of A_{11} and A_{22} depend linearly on L_{12}, which is a portion of the rod domain sequence lacking lysine residues. Its axially projected length, therefore, is ill-defined. In order to compare the results of K5/K14 with K1/K10 in their entirety, it is necessary to use a common value for the length of $L12$. This is a reasonable assumption in view of the very high degrees of sequence homology between the K1 and K5 chains and between the K10 and K14 chains, and thus their likely conformations (Conway & Parry, 1988). Therefore, a combined set of equations relating to the K1/K10 and K5/K14 data sets (here designated K1/K5/K10/K14) was complied in which only unique data were included. The least-squares analysis subsequently carried out on the 39 pieces of data resulted in values for $L1$, $L12$, and $L2$ of 16.27, 13.94, and 5.06 residues, respectively, where each residue has an axially projected length unit, h_{cc}, of 0.1485 nm, typical of an α-fibrous coil–coil rope. With these three values held constant, least squares analyses of the K5/K14 and K1/K10 data were recalculated separately (Table IV). It can be seen that the values of A_{12}, A_{11}, and A_{22} are almost identical for the two molecules, as are the various axial staggers between the coil–coil segments.

Since the repeat length is less than the molecule length (Table IV), a fourth mode of intermolecular alignment, termed A_{CN} (Figure 6), a head-to-tail overlap between parallel molecules, is again confirmed and results in a head-to-tail overlap of about $4.6h_{cc}$ for K5/K14 and $9.4h_{cc}$ for K1/K10. This value is derived from several others, each with its own degree of uncertainty. Consequently, the difference between the two values for K5/K14 KIF and K1/K10 oligomers would seem to lie within the experimental uncertainty. Therefore, there can be little doubt that K1/K10 and K5/K14 molecules are organized in the same way.

DISCUSSION

Induced K5/K14 Cross-Links in Oligomers and KIFs Are the Same. The use of small oligomers as targets for cross-linking studies designed to adduce the lateral organization of neighboring molecules in KIFs is controversial because of an uncertainty about the assembly competence of the oligomers (Stewart, 1993), that is, whether the molecule alignments in the oligomers are in fact the same as those adopted in KIFs. In a previous study (Steinert et al., 1993) we showed that while disulfide-bond cross-linked K1/K10 oligomers are assembly competent, the assembly of the DST cross-linked oligomers could not be documented, presumably because an excessive degree of chemical modification of side-chain lysines at 0.6 mM DST inhibited subsequent reassembly. In this study with K5/K14 oligomers, we were able to precisely control the degree of chemical modification, so that at 0.4 mM DST the one-, two-, and three-molecule oligomers retained assembly competence with only about 10% lysine modification (Figure 1). In addition, the one-molecule oligomer was directly capable of participating in KIF assembly in vitro only if it was not highly modified (Figure 3). In fact the present data suggest that at 0.6 mM DST about 3 times more lysine side chains are modified per mole (Figure 1A), thereby offering a simple explanation for our earlier work. Moreover, we show here that many of the same lysine partners in cross-links seen in the small oligomers were also recovered from intact K5/K14 KIFs (Table I). Together, these data provide convincing evidence for the physiological significance of the molecule alignments adduced in the KIF.

The Structures of K5/K14 and K1/K10 KIF Have Been Conserved. Perhaps the most compelling evidence for the validity of the data is that the axial parameters defining the antiparallel assembly of K5/K14 molecules are essentially identical to those obtained previously for K1/K10 (Steinert et al., 1993) (Table IV; Figure 6). What uncertainty does exist in calculating, say, the molecular lengths for the K5/K14 and K1/K10 heterodimers lies predominantly with the exact properties of the L_{12} link segment. There are no lysines available for cross-linking in L_{12} in any of the four keratin chains studied so far. Furthermore, few equations derived from the available cross-link data provide sufficient constraints to define $L12$ precisely. These particular sequences are unique in biology (Conway & Parry, 1988; Parry, 1990), and consequently there are few clues as to their conformations and thus their net axial lengths.

Table I: Cross-Links in Human K5/K14 Oligomers and KIFs

	cross-linkage (*heptad position*)	sequence[a]	oligomer size	yield (mol/mol)
		Intramolecular Cross-Links		
1	K5: 1A-17*g*	LASYLDKVR		
		*	1,2,3,F	0.35,0.16,0.24,0.17
	K14: 1A-17*g*	LAYLDKVR		
2	K5: 1B-42*g*	NKYEdqink		
		*	1,2,3,F	0.52,0.60,0.15,0.86
	K14: 1B-42*g*	TKYEtelnlr		
3	K5: 2B-23*e*	NTKHEise		
		*	1,2,3,F	0.67,0.66,1.11,0.61
	K14: 2B-23*e*	evatnseLVQSGKSE		
4	K5: 2B-4*g*	aeSWYQTKYE		
		*	1,2	0.29,0.14
	K14: 2B-4*g*	eWFFTKTE		
5	K5: 1B-89*e*	INFMKFFDae		
		*	1,2	0.29,0.76
	K14: 1B-89*e*	eLAYLKK		
6	K5: 1B-89*e*	INFMKMFFD		
		*	1,2,3,F	0.27,0.14,0.16,0.07
	K14: 1B-90*f*	KKNHEe		
		Intermolecular Cross-Links in Alignment Mode A_{11}		
7	K5: 1A-10*g*	TLNNKFASFIDk		
		*	2,3,F	0.14,0.30,0.23
	K14: 2A-19*g*	MAEKNR		
8	K5: 1B-49*g*	YEDQINKR		
		*	2,3,F	0.47,0.28,0.51
	K5: 1B-49*g*	YEDQINKR		
9	K5: 1B-40*e*	DFKNK		
		*	2,3,F	0.15,0.23,0.19
	K5: 1B-61*e*	ttaeneFVMLKK		
10	K14: 1B-6*f*	DYSPYFKTIEdlr		
		*	2,3,F	0.70,0.53,0.62
	K14: 1B-89*e*	eeLAYLKK		
11	K5: 1B-6*f*	NHFVMLKK		
		*	2	0.17
	K5: 1B-42*g*	NKYEdqink		
12	K5: 1B-61*e*	ttaeNHFVMLKK		
		*	2,3	0.22,0.13
	K14: 1B-42*g*	TKYEte		
13	K5: 1B-89*e*	INFMKMFFDae		
		*	3	0.66
	K14: 1B-6*f*	DYSPYFKTIE		
14	K14: 1B-6*f*	DYSPYFKTIE		
		*	2,3,F	0.57,0.67,0.62
	K14: 1B-90*f*	LAYLKKNHEee		
15	K5: 1A-17*g*	FASFIDKVR		
		*	2,F	0.66,0.36
	K5: 2A-10*e*	siiaeVKAQYE		
16	K14: 1B-14*g*	NKILTATVDNAD		
		*	F	0.21
	K14: 1B-82*e*	quieSLKEelaylk		
		Intermolecular Cross-Links in Alignment Mode A_{22}		
17	K5: 2B-44*e*	aeIDNVKK		
		*	2,3,F	0.18,0.11,0.10
	K5: 2B-83*e*	AKQDmar		
18	K5: 2B-23*e*	NTKHEisemnr		
		*	2,3	0.14,0.08
	K5: 2B-100*a*	VKTR		
19	K5: 2B-44*e*	aeidNVKK		
		*	2,3,F	0.12,0.23,0.25
	K5: 2B-81*c*	ALQKAK		
20	K5: 2A-10*e*	nldldsiiaeVKAQYEe		
		*	3,F	0.17,0.34
	K14: E2-25	TKLMDVDGK		
21	K5: 2B-45*f*	aeIDNVKKQCANLQNAIAD		
		*	2	0.28
	K5: 2B-83*e*	AKQDmar		
		Intermolecular Cross-Links in Alignment Mode A_{12}		
22	K5: L1-6	wtilqeQGTKTVR		
		*	3,F	0.22,0.22
	K5: 2B-83*e*	AKQDm		
23	K5: 1B-49*g*	YEDQINKR		
		*	3,F	0.11,0.37
	K14: 2B-23*e*	evatnseLVQSGKSEiselr		

Table I: (Continued)

	cross-linkage (*heptad position*)	sequence[a]	oligomer size	yield (mol/mol)
		Intermolecular Cross-Links in Alignment Mode A_{12} (continued)		
24	K5: 1B-49*g*	YEDQINKR		
		*	3	0.37
	K5: 2B-23*e*	NTKHEIse		
25	K5: 2B-66*b*	GELALKD		
		*	3	0.10
	K14: 1B-6*f*	YSPYFKTIED		
26	K5: 1A-10*g*	TLNNKFASFIDK		
		*	3,F	0.36,0.20
	K5: 2B-112*f*	laldveIATYKKLLEgeecr		
27	K5: 2A-10*e*	VKAQYE		
		*	3	0.10
	K14: 1B-90*f*	LAYLKKNHE		
28	K14: 1B-82*e*	qieSLKEelaylk		
		*	F	0.41
	K14: 2A-19*g*	mAEKNR		
29	K14: 1B-6*f*	DYSPYFKTIEdlr		
		*	F	0.11
	K5: 2B-71*e*	NKLAEle		
		Crosslinks Involving End-Domain Sequences Only		
30	K14: E2-25	TKVM		
		*	F	0.25
	K14: E2-33	dvhdGKVVSTHE		

[a] Deduced amino acid sequences of cross-links are shown using single-letter code; lowercase letters denote likely additional sequences, based on minor peptide species derived from incomplete V8 digestion.

By integration of the two sets of data, which in effect increases the data base of cross-links for fitting of each of the parameters, we have been able to refine our estimates of the intersegment axial staggers (Table III); these produce only minor changes in those for either set viewed separately. Furthermore, these data show that all three modes of nearest-neighbor molecule alignments (A_{11}, A_{22} and A_{12}) also occur in K5/K14 KIF, as seen previously (Figure 6), and that a fourth key overlap A_{CN} can be deduced between the ends of similarly directed molecules which results in an overlap of about 5–11 residues (Figure 6). In addition, the lengths of the $L1$ and $L2$ links differ little for the three data sets and are consistently estimated from the least squares analyses to be about 16–17h_{cc} and 5–10h_{cc}, respectively (Table III). Link $L12$ is highly variable in length, however (data not shown), depending on which of the cross-link equations are incorporated in the analyses. It must be emphasized that this arises as a result of the few independent constraints placed upon it by the cross-link data. For this reason, the value determined for the combined data set (13.94h_{cc}) was kept fixed, together with those for $L1$ and $L2$, and a further least-squares analysis was then carried out so that a direct comparison could be made between the intersegment staggers, etc., for K1/K10 and K5/K14 (Table IV). The very high sequence homology for $L12$ and $L2$ for K1/K10 and K5/K14 would seem to preclude the possibility that the lengths of these links are different in the two molecules, and hence the assumption that they may be fixed is reasonable.

The net result of the data sets is that K1/K10 and K5/K14 KIFs can be built on surface lattices which are identical within the limits of our cross-linking data (Steinert et al., 1993), implying identity of their three-dimensional conformations as well. The data therefore provide a precise molecular understanding of the observations made many years ago that almost any combination of type Ib and type IIb (so-called "soft" keratin or "cytokeratin") chains will copolymerize in vitro (Steinert et al., 1976; Hatzfeld & Franke, 1985) or in in vivo transfection assays (Guidice & Fuchs, 1987; Kartasova et al., 1993). Our data suggest that any combination of cytokeratin molecules of length about 46 nm can successfully dock together in the four alignment modes confirmed here to form a typical KIF in vitro or in vivo.

The present data showing that K5/K14 KIFs have the same structure as K1/K10 KIFs differs from an earlier report (Coulombe & Fuchs, 1990) on K5/K14 structure. On the basis of the appearance of large "lollipop"-like beads, which were thought to represent amino-terminal sequences of the K5/K14 heterodimer molecules, it was concluded that the basic building block for K5/K14 is a four-chain structure, apparently consisting of a pair of molecules aligned in an A_{12}-like mode. However, other studies have convincingly established that an A_{12} alignment does not occur, and in fact is unstable, in solution at the two-molecule stage of assembly (Potschka et al., 1986; Steinert, 1991b; Steinert et al., 1993) and that the smallest oligomers in solution consist of a pair of molecules aligned in both the A_{11} and A_{22} modes (Steinert, 1991a,b; Geisler et al., 1992; Steinert & Parry, 1993; Steinert et al., 1993). The lollipops seen in the earlier study (Coulombe & Fuchs, 1990) are most likely negative-staining artifacts (Steinert, 1991b).

The Structures of Cytokeratin and Trichocyte KIF Are Different. To date there has been only one report of copolymerization of combinations of chains from "hard" or "trichocyte" keratin with cytokeratin chains to form heterodimer molecules of the kind Ia/IIb and Ib/IIa (Herrling & Sparrow, 1991). However, assembly into higher order structures has not been reported. Detailed examination of the properties of keratin chains reveals that all of their coiled-coil rod domain segments and their $L12$ and $L2$ linkers contain exactly the same numbers of residues and are remarkably homologous (Conway & Parry, 1988; Parry, 1990) and thus likely to be of the same axial length. In comparison to the cytokeratins, however, the linker L1 of trichocyte keratins is different in size and sequence especially with respect to the cysteine content. Thus the axially projected length of the $L1$ link segment of trichocyte keratins is likely to be different, with the result that the exact axial length of the heterodimer molecule would also be different. A detailed surface lattice structure for the trichocyte KIF of the porcupine quill tip has

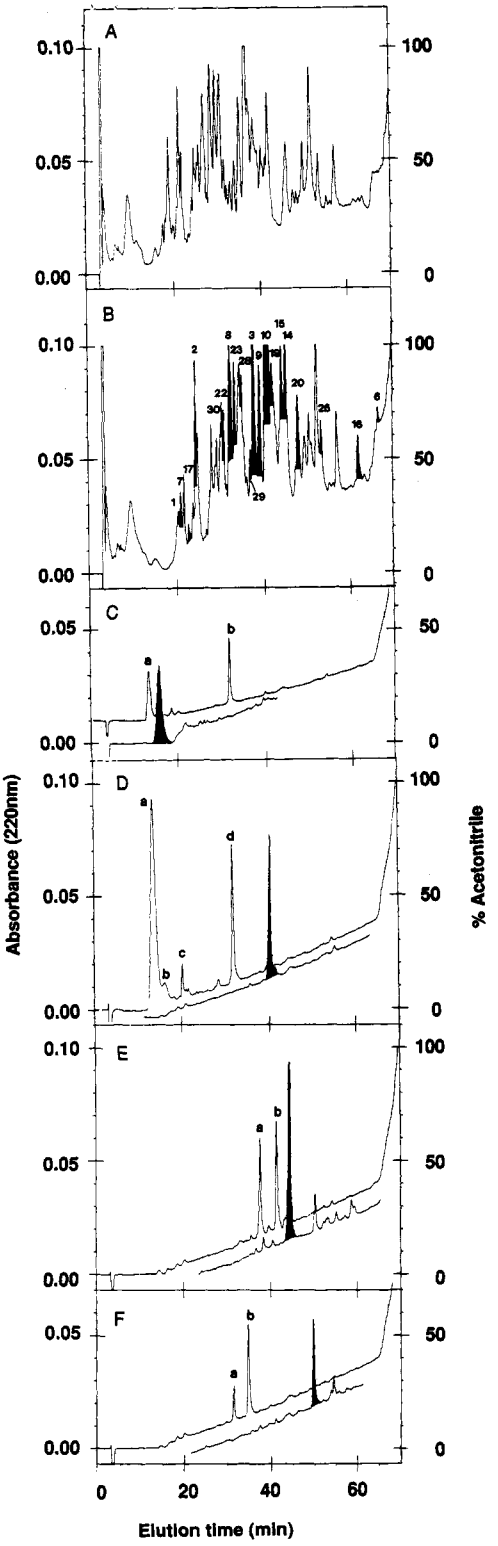

FIGURE 5: HPLC fractionation of cross-linked tryptic and CNBr peptides of human K5/K14 KIF; details are as in Figure 4. (A) Uncross-linked KIF (0.45 mg/mL) cleaved with CNBr and trypsin. (B) Cross-linked KIF (*shaded*). (C-F) Selected profiles obtained in recovery of "arms" of cross-linked peptides from (B) that had been cleaved with V8 protease and then with periodate: (C) peak 7 (shaded), which contains arms from K14 2A-19 (a) and K5 1A-10 (b); (D) peak 3 (shaded), with arms from K5 2B-23 (a,b) and K14 2B-23 (c,d); (E) peak 14 (shaded), with arms from K14 1B-90 (a) and K14 1B-6 (b); and (F) peak 26 (shaded) with arms from K5 1A10 (a) and K5 2B-112 (b).

been determined by low-angle X-ray diffraction methods (Fraser & MacRae, 1983). Interestingly, the repeat length has been measured to be 47 nm, which implies that the molecule

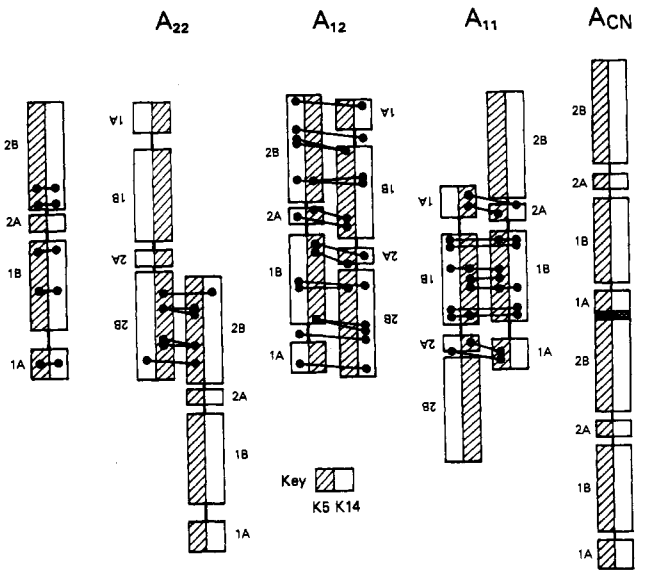

FIGURE 6: Models of molecule alignments detemined from lysine–lysine cross-links induced by DST between K5 (shaded) and K14 (unshaded) chains. The model on the left shows intramolecular cross-links within a K5–K14 heterodimer in which the chains are aligned in parallel and in close axial register. A_{22}, intermolecular cross-links between antiparallel molecules staggered so that their 2B segments are largely overlapped; A_{12}, intermolecular cross-links between antiparallel molecules that are almost exactly overlapped; A_{11}, intermolecular cross-links between antiparallel molecules staggered so that their 1B segments are largely overlapped. The shaded portion of model A_{CN} shows that the last few residues of the 2B rod domain segment overlap the first few residues of the 1A segment of two similarly directed molecules; this overlap is deduced from the analysis of all of the cross-link data.

Table II: Unique K5/K14 Cross-Links Used in the Least-Squares Analyses and Determination of the Equations Used[a]

cross-link	A_{12}	A_{11}	A_{22}	$L1$	$L12$	$L2$	value
K5: L1-6/K5: 2B-83	1	0	0	-0.5	0	0	-4
K5: 1B-40/K14: 2B-23	1	0	0	-1	0	0	-24
K5: 1B-49/K5: 2B-23	1	0	0	-1	0	0	-15
K5: 2B-66/K14: 1B-6	1	0	0	-1	0	0	-15
K5: 1A-10/K5: 2B-112	1	0	0	0	0	0	0
K5: 2A-10/K14: 1B-90	1	0	0	-1	0	1	-6
K5: 1B-49/K14: 2B-23	1	0	0	-1	0	0	-15
K14: 1B-82/K14: 2A-19	1	0	0	-1	0	1	-5
K5: 2B-71/K14: 1B-6	1	0	0	-1	0	0	-10
K5: 1A-10/K14: 2A-19	0	1	0	0	0	1	-112
K5: 1B-49/K5: 1B-49	0	1	0	-1	1	1	-109
K5: 1B-40/K5: 1B-61	0	1	0	-1	1	1	-106
K14: 1B-6/K14: 1B-89	0	1	0	-1	1	1	-112
K5: 1B-61/K5: 1B-42	0	1	0	-1	1	1	-104
K14: 1B-6/K14: 1B-90	0	1	0	-1	1	1	-111
K5: 1B-61/K14: 1B-42	0	1	0	-1	1	1	-104
K5: 1A-17/K5: 2A-10	0	1	0	0	0	1	-114
K14: 1B-14/K14: 1B-82	0	1	0	-1	1	1	-111
K5: 1B-89/K14: 1B-6	0	1	0	-1	1	1	-112
K5: 2B-44/K5: 2B-83	0	0	1	-1	-1	-1	160
K5: 2B-23/K14: 2B-100	0	0	1	-1	-1	-1	156
K5: 2B-44/K5: 2B-81	0	0	1	-1	-1	-1	158
K5: 2B-45/K5: 2B-83	0	0	1	-1	-1	-1	161

[a] The equations are derived as follows: for example, that for the K14: 1B-14/K14: 1B-82 cross-link is given by $(0 \times A_{12}) + (1 \times A_{11}) + (0 \times A_{22}) - (1 \times L1) + (1 \times L12) + (1 \times L2) = -111$.

length is close to 48 nm, assuming that the A_{CN} overlap of about 1 nm is maintained. Furthermore, the surface lattice model (Fraser et al., 1986, 1990) revealed that the axial registration of neighboring molecules is different from that adduced for K1/K10 (Steinert et al., 1993) and K5/K14 KIF. Detailed cross-linking experiments of trichocyte KIF would now seem desirable.

Table III: Intersegment Axial Staggers, in Residues, in K5/K14 and K1/K10 KIFs and a Comparison with Those from an Analysis of All KIF Cross-Link Data

	K5/K14	K1/K10	K1/K5/K10/K14
Δz(1BU,1BD)	−3.63	−4.00	−3.89
Δz(2BU,2BD)	3.75	1.50	2.56
Δz(1BU,2D)	−50.50	−52.97	−50.84
A_{12}	1.50	−1.00	0.43
$L1$	17.00	16.97	16.27
$L2$	10.00	5.63	5.06

Table IV: Calculation of Axial Staggers from Cross-Link Data[a]

structural feature	K5/K14[b,c]	K1/K10[b,c]	K1/K5/K10/K14[c]
molecular length	311.27 (46.22)	311.27 (46.22)	311.27 (46.22)
axial repeat	306.72 (45.55)	301.83 (44.82)	304.44 (45.20)
overlap	4.55 (0.68)	9.44 (1.40)	6.83 (1.01)
Δz(1BU,1BD)	−4.97	−2.33	−3.89
Δz(2BU,2BD)	3.75	1.50	2.56
Δz(2U,2D)	27.81	25.56	26.61
Δz(1BU,2D)	−49.28	−52.73	−50.84
Δz(1BU,1BU)	±77.34 or ±37.35	±84.63 or ±23.97	±80.34 or ±31.71
Δz(2U,2U)	±77.34 or ±37.35	±84.63 or ±23.97	±80.34 or ±31.71
Δz(1BU,2U)	0.25 or −77.09	6.34 or −78.29	2.89 or −77.45
A_{12}	1.99 (0.30)	−1.46 (−0.22)	0.43 (0.06)
A_{11}	−112.70 (−16.74)	−110.06 (−16.34)	−111.62 (−16.58)
A_{22}	194.02 (28.81)	191.77 (28.48)	192.82 (28.63)

[a] Values are in residues (values in parentheses are in nanometers). [b] $L1$, $L12$, and $L2$ were determined for K1/K5/K10/K14 and had the values of $16.27h_{cc}$, $13.94h_{cc}$, and $5.06h_{cc}$, respectively. These values were then held constant for K5/K14 and K1/K10, and the remaining parameters were refined. [c] Measured in multiples of the mean axial rise in a coiled-coil conformation, h_{cc} (0.1485 nm).

Implications for Identity of Molecular Length: Simple Exchange and Integration of KIF Molecules in Preexisting KIF Networks during Differentiation. A variety of recent studies have documented that KIFs in particular (and IFs in general) are highly dynamic structures, often exchanging protein along their lengths in response to a variety of normal cellular events, such as mitosis and locomotion, or experimental manipulation, such as microinjection or treatment with drugs [see Skalli et al. 0(1992) and Vikstrom et al. (1992) for recent reviews]. The available data points to the conclusion that these dynamic exchange events are controlled by phosphorylation/dephosphorylation cycling; that is, a series of specific protein kinases hyperphosphorylate the KIF molecules in the network, promoting localized KIF disassembly, followed later by dephosphorylation by a series of phosphatases, which permits spontaneous reassembly or reassociation onto the KIF network (Erickson et al., 1992; Skalli et al., 1992). Thus a small "soluble" pool of hyperphosphorylated keratins, probably consisting of small oligomers (Steinert, 1991a), undergo these dynamic exchange processes (Miller et al., 1991; Skalli et al., 1992; Vikstrom et al., 1992).

Related to this phenomenon is the question of how the differentiation-specific sets of IF chains become incorporated into the preexisting IF network, such as the changeover from K5/K14 KIF of basal epidermal cells to K1/K10 KIF of suprabasal epidermis. Since there is no evidence for multiple IF organizing centers in these epithelial cells (Goldman & Steinert, 1990), one possible hypothesis (Steinert & Liem, 1990) is that the newly expressed K1/K10 molecules undergo a dynamic exchange process, so that, eventually, the entire KIF network is replaced by, and consists almost entirely of, K1/K10 protein. This hypothesis has been predicated on the following types of data. Recently, it was shown in transfection studies that stable incorporation of the K1 or K10 chain onto the cytoskeleton of epidermal cells in culture requires the preexistence of an intact K5/K14 network (Kartasova et al., 1993). Indirect immunofluorescence and immunogold experiments using chain specific antibodies have shown in transfection assays that the type IV NF-L, NF-M, and NF-H chains associate directly with the preexisting IF network of fibroblasts composed of type III vimentin (Chin et al., 1988; Montiero & Cleveland, 1988; Wong & Cleveland, 1990; Liem, 1993). This means that these type III and type IV chains can copolymerize in vivo. On the other hand, microinjections of single keratin chains or vimentin chains into cultured cells which possess both the type I/II and type III IF networks sort out the protein onto its correct network, indicating that type I/II keratin IFs cannot copolymerize in vivo with type III IFs (Miller et al., 1991; Vikstrom et al., 1992).

However, it is not yet known how these events occur at the molecular level. This is of considerable importance in view of the recent discoveries that simple single point mutations in keratins [reviewed by Epstein (1992), Fuchs (1992), and Steinert (1993)], resulting in the inappropriate substitution of amino acids in key molecular overlap regions (Steinert, 1993; Steinert et al., 1993), are the proximal causes of at least two types of pathology in the epidermis. Rational therapies for the management or cure of these diseases may not be possible without a clear understanding of the fundamental structural priniciples underlying these phenomena. Mutations in either the K5 or the K14 chain cause epidermolysis bullosa simplex, with generalized blistering in the basal layer of the epidermis. Once an affected cell escapes from the basal compartment, a normal KIF cytoskeleton is formed and a normal terminal differentiation program proceeds (Epstein, 1992). In contrast, in epidermolytic hyperkeratosis, mutations in the suprabasal K1 or K10 chain cause dramatic disruption of the KIF network in the cells, leading to an abnormal differentiation program (Fuchs, 1992; Ishida-Yamamoto et al., 1992; Steinert, 1993).

The present data afford a simple explanation for these phenomena. We propose that the dynamic exchange/replacement processes can occur only when the IF molecules and small oligomers of them possess the same conformational alignment and length, as do those of K5/K14 and K1/K10.

It remains to be seen whether the reason for the coassembly of type III and IV chains in vivo is also due to their similar molecular alignments and whether differences in the alignments between type I/II keratin and type III vimentin IF molecules underlie their assembly incompatibility.

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