Interaction *in vitro* of Non-Epithelial Intermediate Filament Proteins with Histones

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Dedicated to Professor Erich Hecker on the occasion of his 60th birthday

Intermediate Filament Proteins, Histones, Intermediate Filament Protein-Histone Interaction

Non-epithelial intermediate filament (IF) subunit proteins show a high and specific affinity for core histones at physiological ionic strength. When IF proteins are titrated with a mixture of core histones and linker histone H1, in general the latter is totally excluded from complexation and in the adducts formed the moderately-arginine-rich histones H2A and H2B are progressively replaced by the very-arginine-rich histones H3 and H4. At histone saturation, 2 molecules of non-neuronal IF protein bind 1 histone H1 molecule or 8 core histone molecules, whereas due to its glutamic acid rich, C terminal extensions one dimer of the 68 kD neurofilament protein associates with 3 molecules of histone H1 or 24 molecules of core histones. The salt stability of the insoluble association products is dependent on the amount and arginine content of the constituent histone species. Removal of the non-α-helical N- and C-terminal polypeptides from IF proteins by partial chymotryptic digestion does not affect their histone-binding characteristics. Since core histones are only partially inactivated by limited tryptic digestion, they also appear to react through their α-helix-rich central domains; the limit peptide derived from histone H1 is completely inactive at physiological ionic strength. Affinity chromatography of rod domains of IF proteins on core histone-Sepharose 4B and of histones and their limit peptides on vimentin-Sepharose 4B has shown that the interactions involving fractions of histones H3 and H4 are extremely resistant to salt and can be dissociated only with arginine or salt under denaturing conditions. In general, the experimental results revealed close parallels between the association of histones with IF proteins and their interaction with DNA.

Introduction

Intermediate filaments represent a class of intracellular proteinaceous structures whose biological role is still totally unknown. Based on electron and immunofluorescence microscopic observations, they have been suggested to be involved in several structural aspects of eukaryotic cells, together with microfilaments and microtubules particularly in the construction of the cytoskeleton (for reviews on intermediate filaments, see [1–4]). In this context, the view that they act as mechanical integrators of cytoplasmic space [5] has found widest acceptance. However, recent experimentation has cast serious doubts on this notion and shown that the function of intermediate filaments, at least in eukaryotic cells cultured *in vitro*, is much more subtle than hitherto presumed. Thus, microinjection of intermediate filament-specific antibodies into cultured vertebrate cells caused no observable effects on the morphology and physiological activities of these cells [6–8]. Moreover, during early embryogenesis there are certain cell types that are devoid of cytoplasmically extended intermediate filament meshworks [9–12], just as there are several established cell lines which *in vitro* proliferate in the total absence of intermediate filaments [13–16].

Since it appears doubtful that the major function of intermediate filaments lies in their cytoplasmic distribution as three-dimensional networks serving structural purposes, it might be postulated that not the filaments as such but rather their subunit proteins, notably their posttranslationally modified forms, are charged with important and specific functions in eukaryotic cells. In default of any hint as to the intracellular localization of these functions, one strategy to substantiate the above contention will certainly be the *in vitro* screening of IF subunit proteins and their posttranslational derivatives for their potentials to specifically interact with other cellular

Abbreviations: CP, core peptide; CT, carboxy terminus; EAT, Ehrlich ascites tumor; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; IF, intermediate filament; NF, neurofilament; NFP, neurofilament protein; SDS, sodium dodecylsulfate.

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constituents. Following this line, we have recently demonstrated that all non-epithelial IF subunit proteins have high affinities for single-stranded nucleic acids, in most cases especially for single-stranded DNA [17–20]. In conjunction with the high susceptibilities of such proteins to Ca^{2+}-dependent posttranslational modification (Ca^{2+}-activated proteinase: [21–26]; Ca^{2+}-activated transglutaminase: [27]) and the physical association of intermediate filaments with Ca^{2+}-sequestering membrane systems (for a review, see [3]), these observations have led to the hypothesis that IF proteins are involved in the transmission of signals from the cell periphery to the nucleus and that their biological function is eventually a nuclear one [3, 28, 29].

Accepting the premise that, after activation through posttranslational modification, IF proteins associate with intranuclear, DNA-containing structures, it must further be assumed that very likely they also interact with a number of nuclear proteins. Because histones are not only the most abundant and most easily obtainable nuclear protein species but also play a fundamental role in the construction of chromatin and thus in a multitude of DNA-based nuclear events, they present a most interesting object of investigation in the scope of the above, alternative hypothesis concerning the cellular function of IF proteins.

As a first step in the elucidation of a possible functional relationship between histones and various forms of IF proteins, we studied the interaction of these polypeptides *in vitro* under different ionic conditions. Even at unphysiologically high salt concentrations, intact IF proteins and some of their proteolytic degradation products turned out to be highly reactive with histones. The arginine-rich core histones yielded the most stable association products.

Materials and Methods

Materials

Ehrlich ascites tumor (EAT) cells were propagated in suspension culture as described previously [30]. CNBr-Sepharose 4B was purchased from Pharmacia (Uppsala, Sweden), DE52- and CM52-cellulose from Whatman (Maidstone, England). Egg white lysozyme, bovine serum albumin and N,N′-methylene bisacrylamide were obtained from Serva (Heidelberg, FRG), trypsin and chymotrypsin "Enzygel" from Boehringer Mannheim (Mannheim, FRG). Acrylamide was supplied by Eastman Kodak (Rochester, N.Y., USA). Myelin basic protein was prepared by the method of Tigyi *et al.* [31] and purified further by CM52-cellulose chromatography. All other chemicals were of reagent grade and purchased from Roth (Karlsruhe, FRG) and Merck (Darmstadt, FRG).

Preparation of IF proteins

Vimentin was isolated from cultured EAT cells [32], desmin from porcine stomach smooth muscle [33], glial fibrillary acidic protein from bovine brain white matter [34] and neurofilament triplet proteins from porcine spinal cord [20]. All proteins were purified to homogeneity and dissolved in 10 mM Tris-acetate, pH 7.6, 6 mM 2-mercaptoethanol (Buffer I).

Isolation and fractionation of histones

Histones were prepared from cultured EAT cells. Frozen cells were extracted with 10 mM Tris-acetate, pH 7.6, 1 mM EGTA, 4 mM $MgCl_2$, 6 mM 2-mercaptoethanol, 0.5% Triton X-100 (Buffer II) until the supernatant of a low speed centrifugation was clear and colourless. After dissociation of vimentin from the resulting residual cell structures by several extractions with 10 mM Tris-acetate, pH 7.6, 1 mM EGTA, 6 mM 2-mercaptoethanol (Buffer III) [32], the remaining chromatin was shaken for 2 h in 0.25 M HCl. Insoluble material was removed by centrifugation at $30{,}000 \times g_{av}$ for 10 min. Core histones were precipitated by adjusting the supernatant to 5% trichloroacetic acid. Following centrifugation at $30{,}000 \times g_{av}$ for 5 min, histone H1 was recovered from the supernatant by increasing the trichloroacetic acid concentration to 25% and collecting the precipitate by centrifugation at $30{,}000 \times g_{av}$ for 5 min. The pelleted core histones were redissolved in 0.25 M HCl and the fractionation was repeated. Core histones and histone H1, respectively, were dissolved in 10 mM Tris-acetate, pH 7.6, 3 mM EDTA, 6 mM 2-mercaptoethanol, 6 M urea, 100 mM KCl (Buffer IV), dialysed against Buffer IV and purified by CM52-cellulose chromatography employing a 100 to 700 mM KCl gradient in 10 mM Tris-acetate, pH 7.6, 3 mM EDTA, 6 mM 2-mercaptoethanol, 6 M urea (Buffer V) for protein elution. Histone H1 which was essentially pure was dialysed against Buffer I, lyophilized

and finally dissolved in and dialysed against Buffer I. Core histones were treated in the same way except that the lyophilized material was taken up in 6 M urea, 0.6% acetic acid, 6% 2-mercaptoethanol and subjected to further purification by preparative polyacrylamide gradient gel electrophoresis in 6 M urea, 6% acetic acid according to the procedure described by Traub and Boeckman [35]. Protein was eluted from the sliced gels electrophoretically in 0.6% acetic acid, dialysed against Buffer I, lyophilized and finally dissolved in and dialysed against Buffer I.

Proteolytic digestion of IF proteins and histones

For the preparation of α-helical rod domains and non-α-helical, C-terminal polypeptides, vimentin, glial fibrillary acidic protein and separated neurofilament triplet proteins were subjected to limited chymotryptic digestion essentially following the procedure of Geisler *et al.* [36, 37]. Matrix-bound enzyme ("Enzygel") was used for digestion. Purification of the breakdown products was carried out by ion exchange chromatography on DE52-cellulose in Buffer V using a 0 to 500 mM KCl gradient. Under these conditions, good separation of the rod and C-terminal domains of the two smaller neurofilament proteins was achieved. An attempt to prepare larger amounts of the NFP 200 rod domain was not successful. The rod domains of vimentin and glial fibrillary acidic protein were additionally purified by affinity chromatography on arginine methylester-Sepharose 4B as described by Traub and Vorgias [38]. All peptides were concentrated by lyophilization and finally dissolved in Buffer I.

The digestion of vimentin with Ca^{2+}-activated neutral thiol proteinase was performed as described previously [38].

The preparation of histone limit peptides employed partial tryptic digestion of histone H1 and core histones, respectively. The globular fragment of histone H1 was prepared according to Hartmann *et al.* [39]. Core histones were digested at 37 °C in 2 M NaCl at a substrate concentration of 1 mg/ml and a substrate/trypsin ratio of 500:1 essentially as described by Weintraub *et al.* [40]. The trichloroacetic acid (5%)-precipitated peptides were dissolved in 6 M urea, 0.6% acetic acid, 6% 2-mercaptoethanol and subjected to purification by preparative polyacrylamide gradient gel electrophoresis in 6 M urea, 6% acetic acid [35].

Titration of IF proteins and their rod domains with histones

In general, 60 μg portions of IF protein were mixed with increasing amounts of a histone H1-core histone mixture in 200 μl of Buffer I containing additional 150 mM KCl at 0 °C. To avoid aggregation of IF proteins under these ionic conditions, the reaction mixtures were immediately centrifuged at $20{,}000 \times g_{av}$ for 5 min. While the pellets were directly dissolved in 100 μl SDS-sample buffer [30], supernatant proteins were precipitated with 25% trichloroacetic acid and, after washing with acetone, also dissolved in 100 μl SDS-sample buffer. In several experiments, supernatant proteins were directly mixed with 5× SDS-sample buffer. A 20 μl portion of each sample was subjected to SDS-polyacrylamide gradient slab gel electrophoresis [30]. Titration experiments employing histone limit peptides, lysozyme, myelin basic protein and histone H1 and core histones separately were carried out in the same way.

Influence of the salt concentration on aggregate formation

60 μg portions of IF protein were usually allowed to react with 30 μg histone H1, core histones, core histone limit peptides, lysozyme and myelin basic protein, respectively, at increasing KCl concentrations (0 to 500 mM KCl) as specified above and in the figures of the "Results" section. In the case of the histone H1 limit peptide, 150 μg peptide was used per 200 μl reaction mixture. Experiments employing free arginine·HCl and lysine·HCl instead of KCl were carried out identically.

Affinity chromatography

The preparation of affinity matrices from CNBr-activated Sepharose 4B was performed according to the manufacturer's instructions. Vimentin was dialyzed against 10 mM $NaHCO_3$ (pH 9) and covalently coupled to CNBr-activated Sepharose in the same buffer by shaking at room temperature for 24 h. The affinity matrix was extensively washed with Buffer V and finally equilibrated with Buffer I. The coupling of histone H1 and core histones, respectively, to Sepharose 4B followed the same procedure. Further experimental details are given in the legends to the figures of the "Results" section. Salt gradients were monitored by conductivity measurement.

Other procedures

SDS-polyacrylamide gradient slab gel electrophoresis was conducted as described by Egberts *et al.* [30]. Proteins were quantitated by scanning of the Coomassie Brilliant Blue-stained gels at 590 nm and integration of the band areas employing a Gilford 2600 spectrophotometer. To obtain absolute values, the results were compared with a calibration curve which was obtained with respective protein solutions of known concentration. Protein concentrations were determined either by using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories GmbH, München, FRG) with crystalline bovine serum albumin and purified histones as the standards or by weighing in purified proteins and peptides. Polyacrylamide gradient slab gel electrophoresis in the presence of 6 M urea, 6% acetic acid was carried out as specified by Traub and Boeckmann [35].

Results

The present investigation was initiated by our studies on the protein composition of residual cell structures which remain after Triton X-100 extraction of vertebrate cells [15, 41]. Hydrochloric acid treatment of Triton cytoskeletons derived from cultured EAT cells dissolved their major protein constituents, nuclear histones and vimentin (Fig. 1b). Upon neutralization of the acidic extract, vimentin was quantitatively precipitated by the core histones H2A, H2B, H3 and H4. Surprisingly, the linker histone H1 as the most basic protein species among the 5 histone classes was totally excluded from aggregation with vimentin and appeared, together with excess core histones, in the supernatant fraction after low speed centrifugation (Fig. 1a). As illustrated in Fig. 1, the solubilization-neutralization cycle could be repeated many times without effectuating changes in the vimentin-core histone ratio of the precipitates, thus demonstrating the high affinity of core histones for the IF protein. Encouraged by these earlier findings, we undertook a detailed study of the interaction of non-epithelial IF subunit proteins with purified histone H1 and core histones.

Reaction of non-epithelial IF proteins with histone H1 and core histones at physiological ionic strength

When individual IF proteins were reacted with increasing amounts of a histone H1-core histone mixture at physiological ionic strength and pH (150 mM KCl, pH 7.6), in general already low quantities of histones were sufficient to precipitate all filament protein (Fig. 2a–f and 3a, b). Precipitated and soluble proteins were separated by low speed centrifugation and quantitated by scanning of their SDS-polyacrylamide gel electrophoresis profiles. It is evident from Fig. 2 that, in the presence of core histones, histone H1 was almost completely prevented from reacting with vimentin, desmin and glial fibrillary acidic protein. Only when the filament proteins were in considerable molar excess over histones, small quantities of histone H1 were incorporated into the aggregates. Another striking feature of the titration curves presented in Fig. 2 was that, whereas at low histone concentrations all 4 core histone species reacted almost equally well with non-neuronal IF proteins, at high core histone concentrations the very-

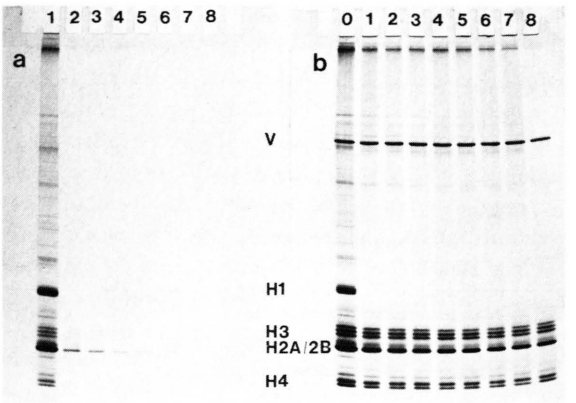

Fig. 1. Association of vimentin with core histones after neutralization of a hydrochloric acid extract of Triton X-100-resistant residual cell structures derived from cultured EAT cells. Triton cytoskeletons prepared as described in "Materials and Methods" were extracted with 0.25 M HCl by shaking at 0 °C for 2 h. The electrophoresis profile of the solubilized protein is shown in slot 0 of panel (b). After neutralization of the extract with 10 M KOH, precipitated material was pelleted by low speed centrifugation. The protein composition of the precipitate is shown in slot 1 of panel (b), that of the supernatant in slot 1 of panel (a). The solubilization-neutralization cycle was repeated 7 times (slots 2 to 8 of panels a and b) by dissolving the precipitates in 0.25 M HCl, neutralization of the resulting protein solutions with KOH and sedimentation of the precipitates by low speed centrifugation. The protein profiles of the precipitates are depicted in panel (b), those of the soluble protein fractions in panel (a). Polyacrylamide gradient slab gel electrophoresis in 6 M urea, 6% acetic acid was carried out as described previously [35]. V, vimentin.

arginine-rich histones H3 and H4 were the predominant histone species being complexed by the filament proteins. Identical results were obtained when the titration experiments were carried out with core histones alone. It should be noted that because of the low electrophoretic resolution of histones H2A and H2B both protein species were quantitated together. However, whenever changes in the histone H2A/H2B complement of IF protein-histone aggregates occurred, *e.g.* during the course of titration experiments or at increasing ionic strength, histone H2B was always the species with the lower affinity for IF proteins (see also Fig. 11).

The situation was substantially different in the case of the separated neurofilament triplet proteins. Fig. 3 shows that with increasing molecular weight these proteins progressively lost the capability to associate with histones. While in comparison with non-neuronal IF proteins (Fig. 2) the 68 kDa neurofilament (NF) protein (Fig. 3a, b) had an even higher capacity to bind histones, the 145 kDa NF protein (Fig. 3c, d) reacted only very reluctantly with histones and the 200 kDa NF protein (Fig. 3e, f) was completely inactive. Moreover, as far as neurofilament triplet proteins were found to react with histones, they also picked up considerable amounts of histone H1 from histone H1-core histone mixtures. However, histone H1 was increasingly replaced by core histones when larger quantities of histone mixture were made available to the NF proteins. Otherwise, the NF proteins showed the same behavior as non-neuronal IF proteins in that they preferentially associated with histones H3 and H4 in the presence of an excess of histone H1-core histone mixture.

The fact that the association products obtained from different IF proteins and a histone H1-core histone mixture were virtually devoid of histone H1 does not mean that IF proteins have no affinity for histone H1. As shown in Fig. 4, histone H1 actually had a high capacity to interact with IF proteins at physiological ionic strength provided the reaction

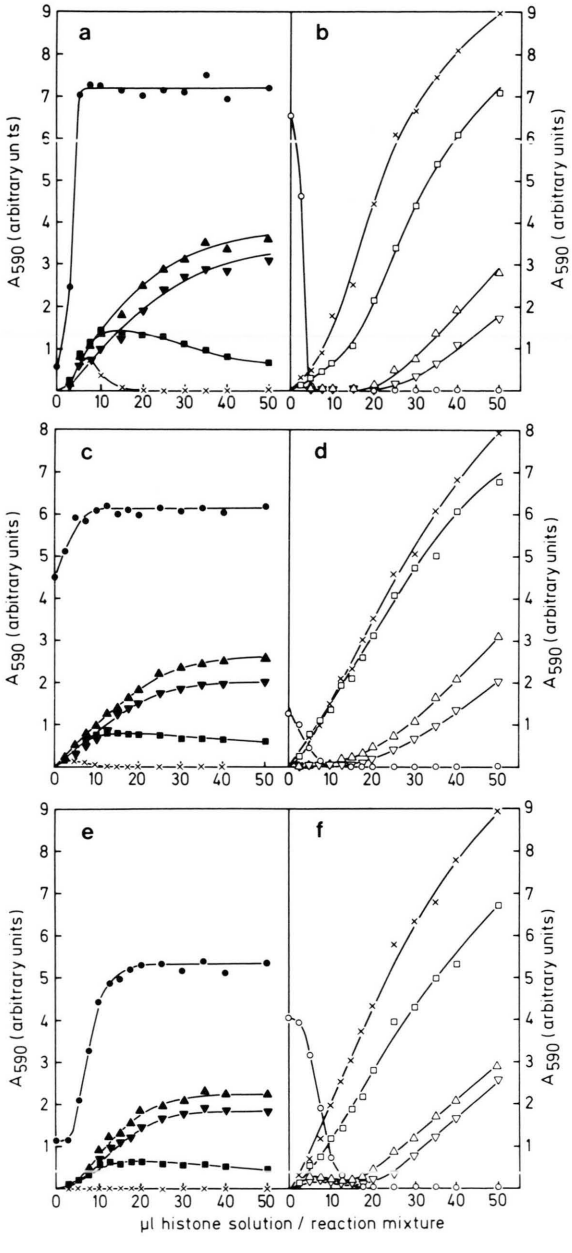

Fig. 2. Titration of vimentin (a, b), desmin (c, d) and glial fibrillary acidic protein (e, f) with a histone H1-core histone mixture at 150 mM KCl. The left hand panels (a, c, e; closed symbols) represent the protein compositions of the pelleted aggregates, the right hand panels (b, d, f; open symbols) those of the corresponding supernatant fractions. The histone solution used for titration contained 1 mg histone H1 and 2 mg core histones/ml Buffer I. The volume of each reaction mixture was 200 µl and contained, in addition to increasing quantities of histones, 60 µg IF protein. Symbols: IF protein (●, ○); histone H1 (x); histones H2A/H2B (■, □); histone H3 (▲, △); histone H4 (▼, ▽). Since desmin has an exceptionally strong tendency to aggregate at higher ionic strength, most of the filament protein was already pelleted in the absence of histones (c, d). Protein analysis was performed by SDS-polyacrylamide gradient slab gel electrophoresis [30] and scanning of the Coomassie Brilliant Blue-stained gels at 590 nm. For further experimental details, see "Materials and Methods".

proceeded in the absence of core histones. It is also apparent from the titration curves of Fig. 4 that the 68 kDa NF protein (Fig. 4b) bound substantially greater amounts of histone H1 than vimentin (Fig. 4a). On the other hand, the 145 kDa (Fig. 4c) and 200 kDa (data not shown) NF proteins did not interact with histone H1 at physiological ionic strength even when histone H1 was present in large excess.

To gain more information on the specificity of the reaction of IF proteins with histones, other basic proteins with molecular weights similar to those of histones were included in the present investigation. Fig. 5a shows that egg white lysozyme was very inefficient in aggregating with vimentin at 150 mM KCl, while myelin basic protein possessed a considerably higher reaction potential (Fig. 5b). However, in comparison with core histones approximately 15 times more myelin basic protein was needed to quantitatively precipitate vimentin. Similar results were obtained with glial fibrillary acidic protein and the 68 kDa NF protein (data not shown). The 145 kDa and 200 kDa NF proteins, however, did not react with lysozyme and myelin basic protein at physiological ionic strength (data not shown).

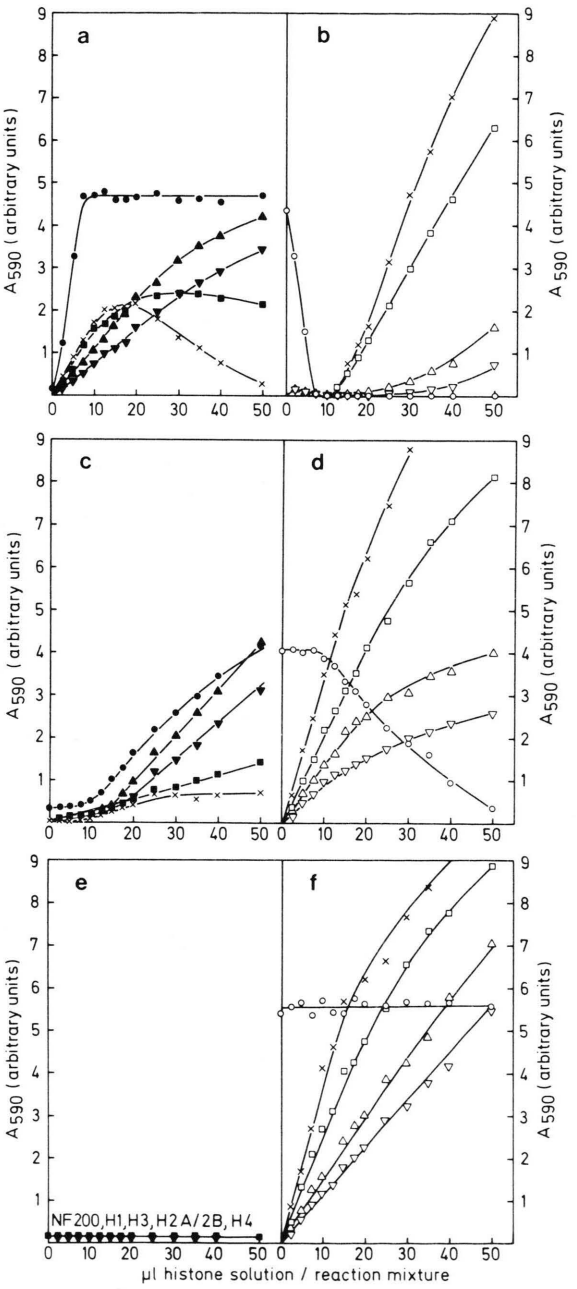

Fig. 3. Titration of NFP 68 (a, b), NFP 145 (c, d) and NFP 200 (e, f) with a histone H1-core histone mixture at 150 mM KCl. The left hand panels (a, c, e; closed symbols) represent the protein compositions of the pelleted aggregates, the right hand panels (b, d, f; open symbols) those of the corresponding supernatant fractions. The histone solution used for the titration of NFP 68 contained 1 mg histone H1 and 2 mg core histones/ml Buffer I; NFP 145 and NFP 200 were titrated with a solution containing 2 mg histone H1 and 3 mg core histones/ml Buffer I. All reactions were carried out with 60 µg portions of NF protein. Symbols: NF protein (●, ○); histone H1 (x); histones H2A/H2B (■, □); histone H3 (▲, △); histone H4 (▼, ▽). Protein analysis was carried out employing SDS-polyacrylamide gradient slab gel electrophoresis [30] and scanning of the Coomassie Brilliant Blue-stained gels at 590 nm. For further experimental details, see "Materials and Methods".

Stoichiometry of IF protein-histone interaction

It appears from Fig. 2 and 3 that at 150 mM KCl the different IF proteins had very similar capacities to bind core histones, with the exception of the 68 kDa NF protein which seemed to complex considerably larger amounts of these proteins. This also holds true for the association of the respective subunit proteins with histone H1 (*cp.* Fig. 4a and Fig. 4b). This qualitative statement was substantiated by determination of the molar ratios of IF proteins and histone H1 and core histones, respectively, in association products formed at histone saturation. While, according to Table I, vimentin, desmin and glial fibrillary acidic protein bound 0.5 molecules of

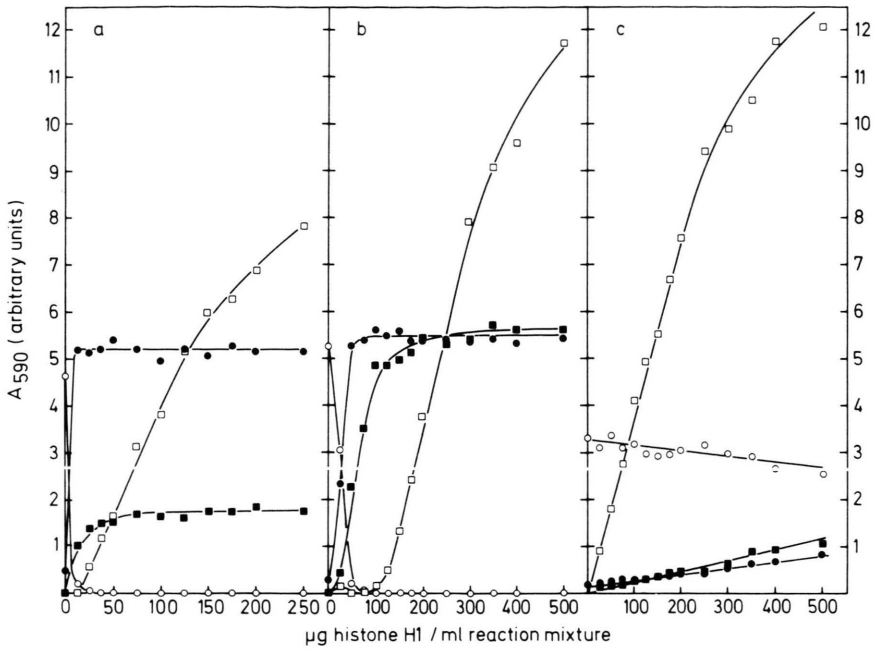

Fig. 4. Titration of vimentin (a), NFP 68 (b) and NFP 145 (c) with histone H1 at 150 mM KCl. 60 µg portions of IF protein (●, ○) were allowed to react with increasing amounts of histone H1 (■, □) in 200 µl Buffer I containing additional 150 mM KCl. After low speed centrifugation, the precipitates and supernatant fractions were analysed by SDS-polyacrylamide gradient slab gel electrophoresis. The protein compositions of the precipitates are represented by closed symbols, those of the supernatants by open symbols. For further experimental details, see "Materials and Methods".

histone H1 and 4 molecules of core histones per molecule, the 68 kDa NF protein picked up 3 histone H1 and 12 core histone molecules, respectively. Since the IF proteins very likely did not react as monomers, a more realistic situation is given by doubling the values listed in Table I. It is clear from Fig. 2 and 3 that at core histone saturation the histone complement of the aggregation products was mainly constituted by the very-arginine-rich histones H3 and H4.

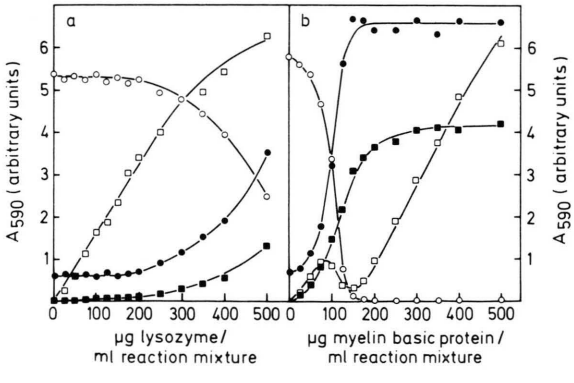

Fig. 5. Titration of vimentin with egg white lysozyme (a) and myelin basic protein (b) at 150 mM KCl. The experimental conditions were the same as those specified in the legends to Fig. 2 to 4. Symbols: Vimentin (●, ○); lysozyme, myelin basic protein (■, □). Closed symbols show the protein compositions of the precipitates, open symbols those of the supernatant fractions.

Table I. Stoichiometry (mol/mol) of histone-intermediate filament protein interaction at histone saturation. 60 µg portions of vimentin, desmin, glial fibrillary acidic protein and NFP 68 were mixed with 100 µg histone H1 and 250 µg core histones, respectively, in 200 µl Buffer I containing additional 150 mM KCl. After low speed centrifugation of the reaction mixtures, the protein compositions of the sediments were determined by SDS-polyacrylamide gradient slab gel electrophoresis and scanning of the Coomassie Brilliant Blue-stained protein bands at 590 nm. Standard curves were obtained by subjecting known amounts of IF proteins, histone H1 and core histones to SDS-polyacrylamide gel electrophoresis under identical conditions. For the calculation of the molar ratios of IF proteins and histones, the following molecular weights were used: Vimentin, 53,500 [47]; desmin, 53,000 [42]; glial fibrillary acidic protein, 50,000 [85]; NFP 68, 65,000 [37]; histone H1, 24,000 [54]; core histones (average), 14,000 [54].

Subunit protein	Histone H1	Core histones
Vimentin	0.79 (0.5, 1?)	4.48 (4)
Desmin	0.56 (0.5)	4.2 (4)
GFAP	0.52 (0.5)	3.85 (4)
NFP 68	2.8 (3)	11.58 (12)

Reactive domains of IF proteins and histones

Knowing the secondary or domain structure of non-epithelial IF proteins [36, 37, 42–47] and histones (for reviews, see [48, 49]), it was of particular interest to determine those structural regions of both protein classes that are responsible for the associations described above. Vimentin, glial fibrillary acidic protein and the 68 kDa and 145 kDa NF proteins were partially digested with chymotrypsin [36, 37] to obtain their α-helical rod domains. When these polypeptides were titrated with a histone H1-core histone mixture at physiological salt concentration (Fig. 6), they behaved very similar to intact non-neuronal IF proteins (*cp.* Fig. 6 and Fig. 2). In all cases examined, the rod domains associated preferentially with histones H3 and H4 and only at lower histone concentrations a comparable interaction with histones H2A and H2B occurred. It was particularly striking that the 145 kDa NF protein which originally showed only reduced reactivity with core histones was considerably activated by removal of its non-α-helical N- and C-terminal polypeptides (Fig. 6c). Moreover, partial chymotryptic digestion of the 68 kDa NF protein abolished its capacity to interact with histone H1 in the presence of core histones (*cp.* Fig. 6b and Fig. 3a). Thus far, we were unable to prepare the NFP 200 rod domain in reasonable quantities; as reported by Geisler *et al.* [37], it is very sensitive to further chymotryptic digestion. However, there are indications that the smaller, α-helical degradation products resulting from such digestions also react with histones in a manner similar to that observed with intact rod domains of the two smaller NF proteins (data not shown). It is clear from these observations that the strong capacity of non-epithelial IF proteins to interact with core histones is a characteristic property of their α-helical rod domains. The partial to total inactivity of the 145 kDa and 200 kDa NF proteins might be due to folding back of their long C-terminal extensions [37, 43, 44] onto their reactive rod domains. A similar decrease in activity of NF triplet proteins with increasing molecular weight was observed concerning their affinity binding to arginine methylester-Sepharose 4B [38] and their competence in filament assembly from separated subunit proteins [50–52].

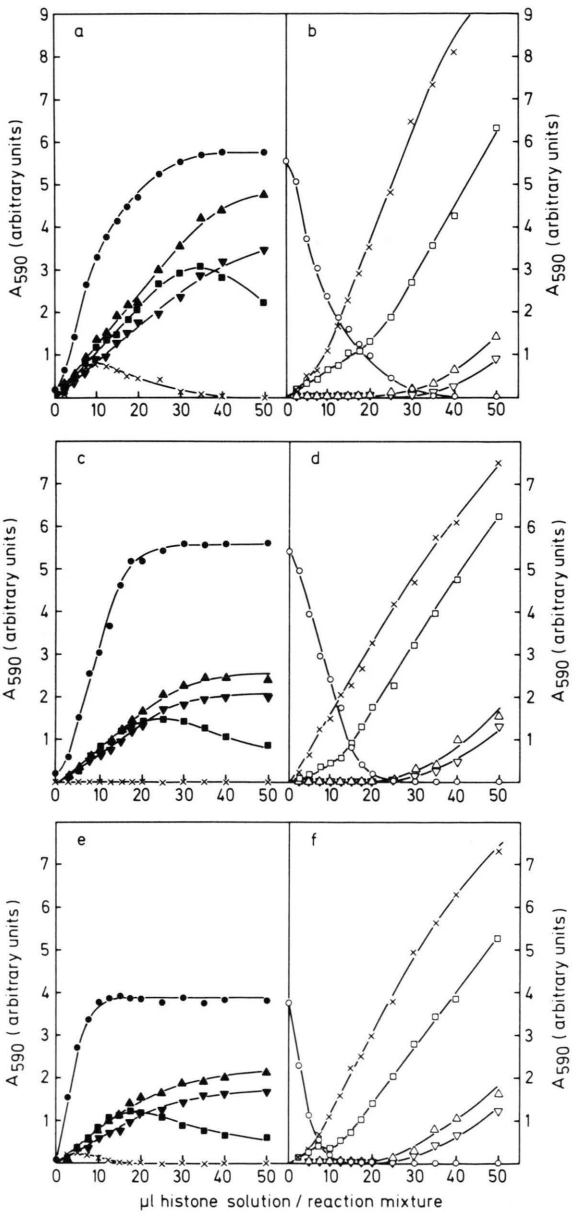

Fig. 6. Titration of α-helical rod domains derived from glial fibrillary acidic protein (a, b), NFP 68 (c, d) and NFP 145 (e, f) with a histone H1-core histone mixture at 150 mM KCl. The left hand panels (a, c, e; closed symbols) show the protein compositions of the precipitates, the right hand panels (b, d, f; open symbols) those of the corresponding supernatant fractions. 60 µg portions of peptides derived from NFP 68 and NFP 145 and 80 µg portions of peptide obtained from glial fibrillary acidic protein were mixed with increasing volumes of a histone solution containing 1 mg histone H1 and 2 mg core histones/ml Buffer I in a final volume of 200 µl. Symbols: Rod domains (●, ○); histone H1 (x); histones H2A/H2B (■, □); histone H3 (▲, △); histone H4 (▼, ▽). For experimental details, see the legend to Fig. 2 and "Materials and Methods".

In an attempt to localize the histone-binding site on the α-helical rod domain of IF proteins, vimentin was digested with Ca^{2+}-activated neutral thiol proteinase which is known to attack this region during longer incubation times [21]. The digestion products were subjected to salt gradient affinity chromatography on histone H1- and core histone-Sepharose 4B, respectively. However, none of the smaller degradation products originating from the rod domain was preferentially retained by either column; although an inverse relationship between the salt concentration effective in polypeptide elution and the size of the polypeptides was noted. Among the degradation products formed, the rod domain and larger polypeptides bound most firmly to both affinity matrices, suggesting that an intact rod domain is essential for tight histone binding. Since twice as much salt was required for polypeptide elution from core histone-Sepharose 4B than from histone H1-Sepharose 4B, this binding does not appear to rest solely on electrostatic interactions (data not shown).

In order to characterize the reactive domains of histones, the same strategy of partial protein degradation was applied. Controlled digestion of histone H1 and core histones with trypsin yielded so-called "limit peptides" [39, 40, 48] which were used for the titration of IF proteins. As examplarily demonstrated with vimentin, at physiological ionic strength the limit peptide derived from histone H1 was totally incapable of aggregating IF proteins (data not shown), whereas the respective peptides obtained from core histones were still very reactive (Fig. 7). However, on a molar basis, substantially greater amounts of the limit peptides were needed to produce the same effect as observed with intact core histones (*cp.* Fig. 7 and Fig. 2a). These results demonstrate that the unstructured terminal polypeptide of histone H1 is essential for tight interaction of histone H1 with IF proteins and that the terminal regions of the core histones are partially dispensable.

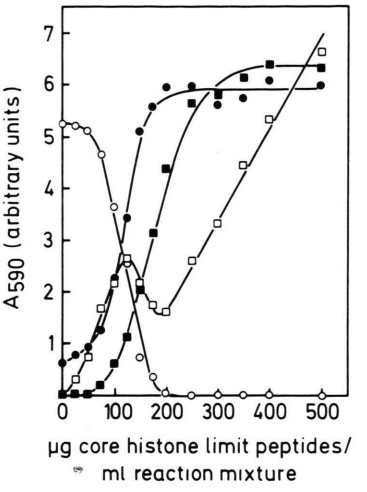

Fig. 7. Titration of vimentin with core histone limit peptides at 150 mM KCl. Limit peptides were prepared from core histones by partial tryptic digestion according to Weintraub *et al.* [40]. 60 µg portions of vimentin (●, ○) were allowed to react with increasing quantities of core histone limit peptides in 200 µl Buffer I containing additional 150 mM KCl. Limit peptides (■, □) in the precipitates and supernatant fractions, respectively, were quantitated as a total. Closed symbols represent the protein compositions of the various precipitates, open symbols those of the corresponding supernatants. For experimental details, see the legend to Fig. 2 and "Materials and Methods".

Influence of the salt concentration on the interaction of IF proteins with histones

Further information on the specificity of the interaction of IF proteins with histones was expected from the salt stability of the resulting association products and from the effect of increasing ionic strength on aggregate formation, respectively. In a first set of experiments, individual IF proteins were allowed to react with a constant and limited amount of histone H1 or core histones at increasing KCl concentration and the distribution of the different reactants among insoluble aggregates and the soluble compartment was determined by SDS-polyacrylamide gel electrophoresis. The results obtained with vimentin and histone H1 and core histones are graphically presented in Fig. 8 and 9, those obtained with the other IF proteins and their rod domains are summarized in Table II. The comparison of Fig. 8 and 9 shows that the formation of sedimentable aggregates from vimentin and histone H1 was more sensitive to salt than that from vimentin and core histones. Moreover, the very-arginine-rich histones H3 and H4 were distinctly more efficient in producing salt-resistant aggregates with vimentin than the moderately-arginine-rich histones H2A and H2B (Fig. 9). The distribution of IF proteins and their rod domains among aggregates and supernatant fractions always followed very closely that of histones H3 and H4. Although the differences in the KCl concentration at which individual histone species still formed salt-stable as-

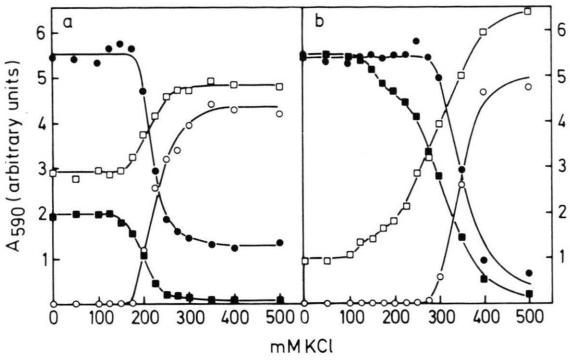

Fig. 8. Influence of the KCl concentration on the aggregation of histone H1 with vimentin (a) and NFP 68 (b). A constant amount of histone H1 (30 μg) was allowed to react with a fixed quantity of vimentin and NFP 68 (60 μg), respectively, in 200 μl Buffer I of increasing KCl concentration. The distribution of IF proteins (●, ○) and histone H1 (■, □) among aggregates (closed symbols) and soluble compartment (open symbols) was determined employing the standard analytical procedure (see the legend to Fig. 2 and "Materials and Methods").

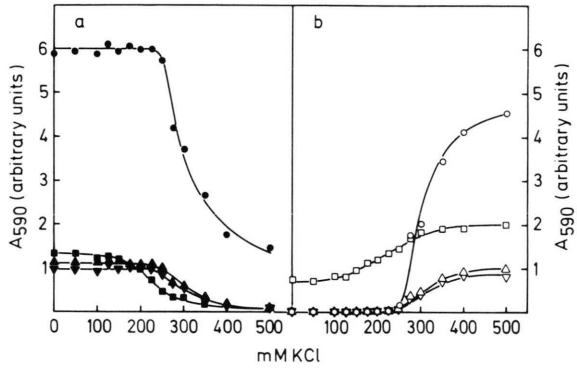

Fig. 9. Influence of the KCl concentration on the aggregation of vimentin with core histones. Panel (a) (closed symbols) shows the protein compositions of the insoluble aggregates, panel (b) (open symbols) those of the corresponding supernatant fractions. 60 μg portions of vimentin were mixed with 30 μg portions of core histones in 200 μl Buffer I of increasing KCl concentration. Symbols: Vimentin (●, ○); histones H2A/H2B (■, □); histone H3 (▲, △); histone H4 (▼, ▽). For experimental details, see the legend to Fig. 2 and "Materials and Methods".

sociation products with IF proteins were relatively small, they were nevertheless significant and observed throughout with all non-epithelial IF proteins examined (Table II). They reflect the differential efficiencies of the various histone species to aggregate IF proteins when allowed to compete for them at 150 mM KCl (Fig. 2 and 3).

Table II. KCl concentration (mM) causing 50% inhibition of the aggregation of IF proteins with histone H1, core histones, lysozyme and myelin basic protein. The half point KCl concentrations were taken from precipitation curves as presented in Fig. 8 and 9. The distributions of IF proteins and their core domains (CDs) followed closely those of histones H3 and H4. The experimental conditions were the same as those described in the legends to Fig. 8 and 9 and in "Materials and Methods".

IF protein	Histone				Lyso-zyme	Myelin basic protein
	H1	H2A/ H2B	H3	H4		
Vimentin	200	220	310	310	60	190
Desmin	200	220	320	320		
GFAP	150	200	230	230		
NFP 68	300	230	250	250		
NFP 145	120	30	30	30		
NFP 200	40	15	15	15		
Vimentin-CP	180	230	350	350		
GFAP-CP	150	200	300	300		
NFP 68-CP	125	175	250	250		
NFP 145-CP	175	220	360	360		

As can be seen from Table II, there were also exceptions to this general behavior of IF proteins; they were particularly observable with NF proteins. In comparison to the relatively low salt resistance of complexes derived from non-neuronal IF proteins and histone H1, that of aggregates obtained from NFP 68 and histone H1 was substantially higher (see also Fig. 8). It was also greater than the stability of the association products formed from NFP 68 and core histones. The same situation was observed with the two larger NF proteins. These differences are very likely due to the presence of long, glutamic acid-rich C-terminal domains in the NF proteins [37, 43, 44] to which the lysine residues of histone H1 might have a special affinity. At any rate, their removal by partial chymotryptic digestion (production of rod domains) brought the situation back to normal (Table II).

Whereas the detachment of the non-α-helical terminal domains from non-neuronal IF proteins did not exert any major effect on the salt-stabilities of the IF protein-histone aggregates (Table II), the limited proteolytic digestion of histone H1 and core histones, on the other hand, was rather influential. Already at 50 mM KCl, aggregate formation from vimentin or NFP 68 and the limit peptide derived from histone H1 was inhibited by 50%, although in comparison to intact histone H1 (Table II) a large excess of the globular histone fragment was used.

The corresponding value obtained with a normal concentration of core histone limit peptides was 160 mM KCl. While the value of 60 mM KCl for the 50% inhibition of complex formation from vimentin and lysozyme (Table II) was expected, the 190 mM KCl determined for vimentin-myelin basic protein adducts (Table II) turned out to be rather high; it is very likely due to the high arginine content of myelin basic protein (10.5%) [53]. These data are compatible with results from titration experiments conducted at physiological ionic strength (Fig. 5).

From the results reported thus far, it is evident that among the various histone species those rich in arginine preferentially interact with IF proteins. It was tempting to speculate, therefore, that arginine residues of the histone molecules play an essential role in the association reactions described above. To prove this, the formation of histone-IF protein adducts was carried out in the presence of increasing concentrations of arginine·HCl and lysine·HCl, respectively. The concentrations of both amino acids effectuating inhibition by 50% of the association of vimentin with different histone species are listed in Table III. They show that on a molar basis arginine·HCl, but surprisingly lysine·HCl also, was slightly more effective in preventing aggregate formation than KCl. However, it should be taken into account that the electric conductivity of arginine·HCl and lysine·HCl solutions is 2.5 times lower than that of KCl solutions of equal concentration.

Table III. Arginine and lysine concentration (mM) causing 50% inhibition of the aggregation of vimentin with histone H1 and core histones. The experimental conditions were exactly the same as those described in the legends to Fig. 8 and 9, except that arginine·HCl and lysine·HCl instead of KCl were used.

	Histone H1	H2A/H2B	H3	H4
Arginine·HCl	150	140	220	220
Lysine·HCl	190	190	260	260

Affinity chromatography

Although the precipitation of IF proteins and their rod domains by histones is quickly inhibited beyond the half point KCl concentrations listed in Table II, it is nevertheless possible that at higher ionic strength soluble histone-IF protein adducts exist which are stabilized by non-electrostatic bonds. A convenient means to test this possibility is affinity chromatography of IF proteins on histone matrices. Since intact IF proteins exhibit a strong tendency to aggregate at high ionic strength, they cannot be used for such purposes. However, due to loss of the N-terminal polypeptide their rod domains are soluble even at high salt concentrations and their interaction with histones is almost identical to that of their parental molecules (Fig. 2, 3, 6, Table II). Fig. 10 depicts the elution of vimentin core peptide (vimentin-CP) from core histone- and histone H1-Sepharose 4B. While the polypeptide was eluted from core histone-Sepharose 4B within a broad KCl concentration range with a peak at approximately 0.6 M KCl, it was desorbed from histone H1-Sepharose 4B in a sharp peak at 0.33 M KCl. Surprisingly, a substantial fraction of the rod domain stuck extremely tightly to the core histone affinity matrix so that it could not be eluted with 1 M KCl. It could only be recovered with high salt under denaturing conditions. Similar results

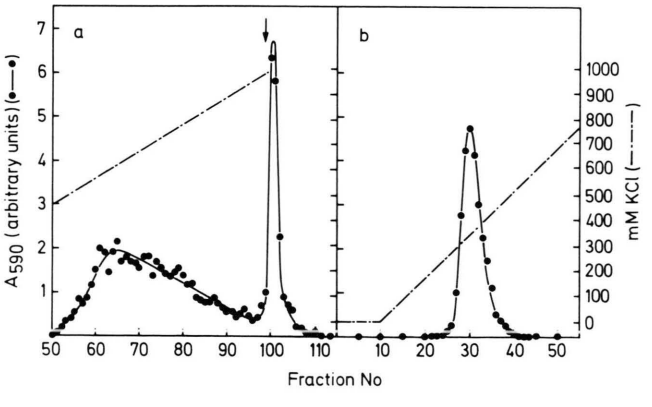

Fig. 10. Affinity chromatography of the rod domain of vimentin on core histone-Sepharose 4B (a) and histone H1-Sepharose 4B (b). 3 mg vimentin core peptide was applied to a 24 × 1 cm core histone- and histone H1-Sepharose 4B column, respectively, and eluted with a 200 ml linear 0 to 1 M KCl gradient in Buffer I. 2 ml fractions were collected. 40 µl of each fraction was mixed with 10 µl 5 × SDS-sample buffer and 20 µl of each mixture was subjected to SDS-polyacrylamide gel electrophoresis. The arrow in panel (a) indicates the position where the column was started to be washed with 6 M urea/1 M KCl (1 M KCl in Buffer V).

were obtained with the rod domains derived from glial fibrillary acidic protein and the neurofilament proteins NFP 68 and NFP 145 (data not shown; see also Table IV). In the latter case, the situation was particularly extreme in that virtually all of the NFP 145 core peptide could only be eluted from core histone-Sepharose 4B with high salt in the presence of urea.

The KCl concentrations effectuating peak elution of the rod domains of IF proteins as well as of the chymotryptic C-terminal fragments of NFP 68 and NFP 145 from histone H1- and core histone-Sepharose 4B are summarized in Table IV. It is obvious that the non-α-helical, glutamic acid-rich C-termini of the two neurofilament proteins [37, 43, 44] have no exceptionally high affinities for histones.

Strong interaction of histones with IF protein was also observed during affinity chromatography of a histone H1-core histone mixture on vimentin-Sepharose 4B. As illustrated in Fig. 11a, histones H1 and H2B left the column at 0.2 M KCl, whereas histone H2A was eluted at 0.4 M KCl. Continued KCl gradient elution released fractions of histones

Table IV. KCl concentration (mM) releasing core and C-terminal domains of various IF proteins from histone H1- and core histone-Sepharose 4B. The experimental conditions are described in the legend to Fig. 11. CP = core peptide (rod domain); CT = carboxyterminal polypeptide. The KCl concentration required for the elution of NFP 145 CP from core histone-Sepharose 4B could not be determined because virtually no material was eluted with a 0 to 1 M KCl gradient.

Polypeptide	Histone H1-Sepharose 4B	Core histone-Sepharose 4B
Vimentin-CP	360	600
GFAP-CP	335	500
NFP 68-CP	310	700
NFP 145-CP	360	–
NFP 68-CT	260	215
NFP 145-CT	230	215

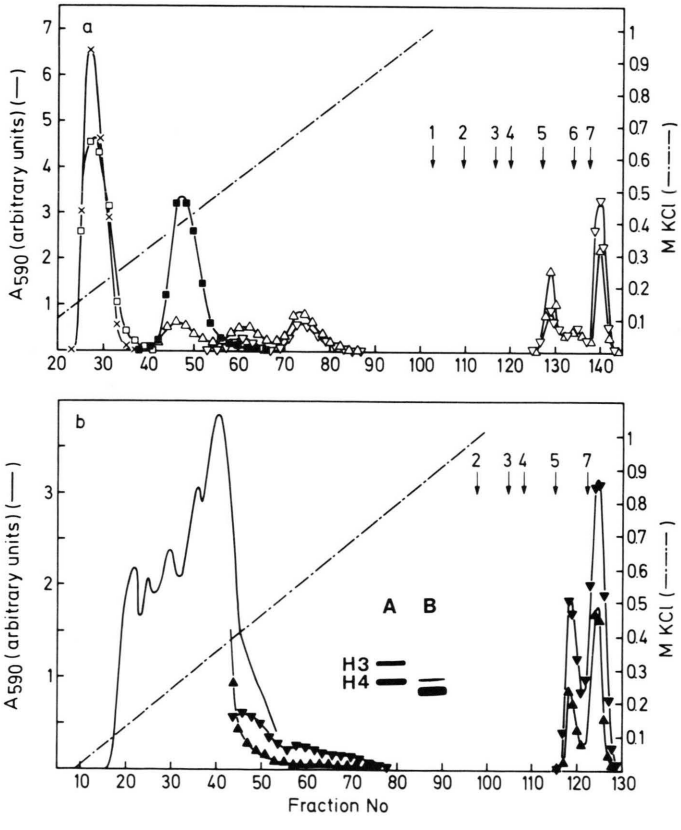

Fig. 11. Affinity chromatography of a histone H1-core histone mixture (a) and core histone limit peptides (b) on vimentin-Sepharose 4B. A histone H1-core histone mixture (1.25 mg histone H1, 5 mg core histones) and core histone limit peptides (7 mg), respectively, were applied to a 19×1 cm vimentin-Sepharose 4B column previously equilibrated with Buffer I. Protein was eluted with a 200 ml linear 0 to 1 M KCl gradient in Buffer I. Thereafter, the column was washed with the following solutions (arrows): Position 1: 1.5 M KCl in Buffer I; position 2: 2 M KCl in Buffer I; position 3: Buffer I; position 4: 0.5 M lysine·HCl in Buffer I; position 5: 0.5 M arginine·HCl in Buffer I; position 6: 1 M arginine·HCl in Buffer I; position 7: 1 M KCl in (urea) Buffer V. 2 ml fractions were collected. Protein of the first 40 fractions of chromatography (a) was precipitated with 25% TCA, protein of all other chromatography fractions with 10% trichloroacetic acid. The precipitates were dissolved in 300 µl SDS-sample buffer and 10 µl of each sample was used for SDS-polyacrylamide gel electrophoresis. Because of insufficient resolution of the individual core histone limit peptides during KCl gradient elution, protein elution was followed by recording of the absorbancy at 280 nm. Symbols: Histone H1 (x); histone H2A (■); histone H2B (□); histone H3 (△); histone H4 (▽); limit peptide of histone H3 (▲); limit peptide of histone H4 (▼). The insert in panel (b) is an SDS-gel electrophoretic comparison of histones H3 and H4 (fraction 140 of chromatography (a)) (lane A) with the limit peptides of histones H3 and H4 (fraction 125 of chromatography (b)) (lane B).

H3 and H4 within a broad KCl concentration range between 0.4 M and 0.7 M KCl. Washing of the affinity column with 2 M KCl or 0.5 M lysine · HCl did not bring about further desorption of histone proteins. However, when the column was washed with 0.5 M or 1 M arginine · HCl, substantial amounts exclusively of histones H3 and H4 could be released, again demonstrating the preferential affinity of vimentin for arginine-rich histones. At present, we do not know why only fractions of histones H3 and H4 show this high binding-affinity for vimentin-Sepharose 4B. Since such a partial retention of protein was also observed during affinity chromatography of the rod domains of IF proteins on core histone-Sepharose 4B (Fig. 10a), heterogeneity of the histone H3 and H4 populations has to be taken into consideration.

Additional information on the association of histones H3 and H4 with vimentin was obtained by affinity chromatography of core histone limit peptides on vimentin-Sepharose 4B (Fig. 11b). Following elution with a KCl gradient, 2 M KCl and 0.5 M lysine · HCl in this order, washing of the column with 1 M arginine · HCl or high salt/urea released two limit peptides which, on the basis of the close similarity of the elution profiles of Fig. 11a and 11b, were tentatively identified as originating from histones H3 and H4. They are electrophoretically compared with their parental molecules in the insert of Fig. 11b.

Discussion

The present investigation has demonstrated that histones have a high and differential affinity for IF proteins at physiological ionic strength. Although this conclusion is to a considerable extent based on the results of precipitation experiments and, thus, must also take into account unspecific, electrostatic interactions, we nevertheless believe the associations described to be of specific nature, for the following reasons: (1) The interaction of IF proteins with histone H1 and core histones is governed by a distinct stoichiometry demonstrating the involvement of only well-defined binding sites of both classes of proteins. (2) Unspecific electrostatic interactions, particularly between IF proteins and the very-arginine-rich core histones H3 and H4, can be largely excluded since the respective adducts are stable in solution of extremely high ionic strength. (3) In the presence of a slight excess of core histones, the linker histone H1 as the most basic protein species [54] among histones does not show affinity for IF proteins and, thus, does not contribute unspecific aggregations even at relatively low ionic strength; although alone it readily reacts with IF proteins and their proteolytic derivatives. (4) Lysozyme as another small basic protein molecule is very inefficient in precipitating IF proteins at physiological ionic strength. Myelin basic protein, on the other hand, has a relatively high reaction potential in this regard, although it is far less effective than core histones despite its richness in arginine residues [53].

Concerning the functional groups and binding domains of IF proteins and histones involved in the above associations, the following conclusions and assumptions can be made on the basis of the present experimental results: The preferential incorporation of arginine-rich histones into aggregates with IF proteins at first glance point to an important role of the arginine residues of the histones. The arginine contents of the various histones [54] (arginine contents: H1: 1.4%; H2B: 6.4%; H2A: 9.3%; H3: 13.3%; H4: 13.7%; arginine/lysine ratios: H1: 0.05; H2B: 0.4; H2A: 0.86; H3: 1.38; H4: 1.27) are in the same order as the affinities of the respective protein species for IF proteins (H1 $\ll$ H2B $<$ H2A $<$ H3 = H4). This conformance is compatible with our previous finding that in their central region IF proteins possess an acceptor site that is capable of accomodating the arginine-rich N-terminus of another IF protein molecule in the process of filament assembly [38]. Indeed, removal of the N- and C-terminal domains from IF proteins by limited chymotryptic digestion does not significantly change their histone-binding potentials, suggesting that the central binding site of IF proteins for arginine-rich polypeptides also plays an at least partial role in the binding of arginine-rich histones. Such an assumption, however, does not seem to agree with the observation that, while filament assembly can be specifically blocked by moderate concentrations of free arginine [38], the aggregation of IF proteins with histones is also partially sensitive to free arginine, but to free lysine as well; although arginine is somewhat more efficient than lysine. At any rate, measured by the conductivity of the reaction mixtures approximately 3 times less arginine · HCl (or lysine · HCl) than KCl is required to prevent the aggregation of IF proteins with histones. Therefore, these associations do not appear to depend solely on electrostatic interactions.

Here, one should also consider that while free lysine might only be active in preventing the precipitation of the primary reaction products, free arginine might additionally inhibit their formation. Support for this contention comes from the results of affinity chromatographies of core domains of IF proteins on histone-Sepharose 4B and of histones on vimentin-Sepharose 4B. They show that the bonds mediating the association of the arginine-rich histones H3 and H4 with IF proteins and their proteolytic derivatives are extremely resistant to free lysine or high salt and only dissociable with free arginine or salt under denaturing conditions. It is reasonable to assume, therefore, that the precipitation of IF proteins by histones is primarily based on the specific association of the very-arginine-rich histones H3 and H4 with the rod domain of the IF proteins. This results in the formation of salt-stable products which, at lower ionic strength, are joined by the moderately-arginine-rich histones H2A and H2B. With decreasing salt concentration, the primary association products aggregate forming precipitates in which arginine as well as lysine residues fulfill a major binding function.

The characterization of the active binding sites of histones mainly encompassed the production of limit peptides by partial tryptic digestion of histones and their affinity chromatography on vimentin-Sepharose 4B. The results show that two of the core histone-derived limit peptides, very likely originating from histones H3 and H4, bind very tightly to the affinity matrix from which they can be eluted only with arginine or salt/urea. Thus, the functional sites essential for the binding of histones H3 and H4 to the central domain of IF proteins must be preserved during tryptic digestion. Since core histones are constructed of a central, globular domain rich in α-helix and unstructured, positively charged C- and N-terminal polypeptides that can be removed by limited tryptic digestion [48, 49], the tight association of both limit peptides as well as of their parental molecules with IF proteins might be envisaged as being mediated through hydrophobic (plus electrostatic) bonds between the α-helical domains of the reactants. Such strong, salt-stable interactions of α-helical regions also provide the basis, for instance, for the formation of coiled-coils in the process of proto- and intermediate filament assembly from IF subunit proteins [55–58]. In addition, this assumption might provide a satisfactory explanation for the finding that intermediate filament assembly responds rather sensitively to free arginine [38], whereas the dissociation of IF protein-histone H3/H4 adducts requires much higher arginine concentrations: The arginine-rich N-terminus of non-epithelial IF proteins does not contain α-helical regions [36, 37, 42–47]. Finally, it is relevant in this connection to refer to the results of hydrophobic interaction chromatography of histones [59, 60] and the extremely high affinity of IF proteins and their proteolytic degradation products for neutral- and phospholipids [61, 62].

As pointed out above, the reaction of histone H1 and core histones with IF proteins has a distinct stoichiometry, although, due to their opposite net charges, the reactants should be expected to associate in a rather unspecific manner. We noted that one molecule of IF protein binds 4 molecules of core histones. This molar ratio, however, does not represent a realistic situation since the IF proteins were offered to the core histones in their protofilamentous, tetrameric form [56]. Because of the high stability of these tetramers, it must be assumed, therefore, that each protofilament unit binds 16 molecules of core histones. On the other hand, in free solution of physiological salt concentration core histones also exist as oligomers, histones H3 and H4 as $(H3-H4)_2$ tetramers and histones H2A and H2B as (H2A–H2B) dimers [59, 60, 63, 64]. On the basis of results from histone octamer [65, 66] and nucleosome [67–70] assembly studies, there is no reason to assume that these oligomers should dissociate into monomers in the process of aggregate formation with IF proteins. Quite to the contrary, it is possible that at physiological ionic strength and moderate core histone concentrations each IF protein tetramer associates with two $(H3-H4)_2$ tetramers each of which is flanked by two (H2A–H2B) dimers in a nucleosome core-like arrangement. However, when core histones are offered to IF proteins in excess, histones H2A and H2B are progressively replaced by histones H3 and H4, probably due to the much higher affinity of the latter for the α-helical rod domain of the IF proteins. Under ideal circumstances, each protofilament would bind 4 $(H3-H4)_2$ tetramers or 2 $(H3-H4)_4$ octamers. Again we note that such $(H3-H4)_4$ octamers are also formed in the process of nucleosome assembly from DNA and excess histones H3 and H4 [68, 71].

Unfortunately, for technical reasons, the physical demonstration of such association products is ex-

tremely difficult. Upon mixing of the components at physiological ionic strength, they immediately precipitate even at 0 °C. One reason for this probably is the twofold symmetry of the histone octamer (or $(H3-H4)_2$ tetramer) [72, 73] and the antiparallel arrangement of 2 IF protein dimers in each protofilament [56]. This bifunctionality of the reactants together with their high affinities for each other gives rise to immediate polymerization reactions. At core histone saturation, each tetrameric protofilament would be in contact with 2 histone octamers (mainly $(H3-H4)_4$ octamers). It should be noted here that at lower core histone concentrations we were able to detect IF protein-histone adducts in the supernatants of low speed centrifugations which contained, beside IF proteins, all 4 core histones (unpublished observations). However, so far, we were not yet able to subject them to a detailed biochemical and electron microscopic analysis.

The results of the present investigation raise the question whether interactions between histones and IF proteins (or more precisely their posttranslational derivatives) also occur *in vivo*. Taken by themselves, they might only be of model character and without physiological significance. However, as long as the true biological role of IF proteins is unknown, these results might be helpful in providing hints as to that function, particularly in conjunction with our recent finding that non-epithelial IF proteins and some of their derivatives are nucleic acid-binding proteins with a preferential affinity for single-stranded DNA [17–20]. In the meantime, we could also demonstrate a high and specific affinity of non-epithelial IF proteins for superhelical DNA (Kühn and Traub, unpublished observations). Taken together, our findings might thus point to important functions of derivatives of IF proteins in the provision of replicable and transcribable DNA in the scope of mitogenesis and gene expression, respectively.

In any event, the similarities between the interaction of histones with IF proteins and their proteolytic derivatives, on the one hand, and the association of histones with DNA [49], on the other, are so striking that they should not escape notice. In both cases, the histones H3 and H4 play a central role. Their limited tryptic digestion has identified their α-helix-rich, central domains as those reactive regions that confer nucleosome-like properties on DNA [48, 49, 74] and bind the arginine-rich histones to the α-helical rod domain of IF proteins. Moreover, the stabilization by DNA of $(H3-H4)_4$ octamers in nucleosome-like structures [68, 71] seems to be paralleled by the binding of 8 molecules of histone H3/H4 to each IF protein dimer. In all these reactions, arginine residues fulfill an essential binding function [75]. Such a coincidence in reactivity might even suggest that IF protein-like material and DNA compete for histones under certain conditions and that, for instance, the former becomes functional in the (at least partial) dissociation of core histones from DNA. There are two ways of how this could be achieved. Firstly, those domains of the core histones which constrain nucleosomal DNA into two physical superhelical turns are transferred to the α-helical rod domains of IF protein derivatives. Simultaneously, in a concerted fashion, the DNA-binding site of the histone acceptor proteins could become active in unwinding double-stranded DNA with the production of single-stranded regions for DNA replication or DNA transcription. Secondly, the arginine-rich, non-structured tail pieces of the core histones which appear to be essential for the stabilization of the higher-order (solenoid) structure of chromatin [48, 49, 76, 77] could associate with the central acceptor site of IF proteins for arginine-rich polypeptides and, thus, lead to the partial decondensation of chromatin. Model building experiments should be helpful in eliciting the feasibility of such reactions from a steric point of view. Currently, we are also investigating whether the rod domains of IF proteins act as nucleosome assembly factors in the transfer of histone octamers, or at least $(H3-H4)_2$ tetramers, from respective protein aggregates to DNA at physiological ionic strength.

We wish to emphasize here that intact IF proteins very likely are not involved in these still hypothetical functions. They occur exclusively in the cytoplasm (for a possible exception, see below) in form of highly insoluble intermediate filaments. Only by activation through posttranslational modification in response to extra- and intracellular stimuli is it supposed that they are released from the filaments and migrate into the nucleus to fulfill their nuclear functions. A detailed description of this novel hypothesis on a possible cellular function of IF proteins has been presented elsewhere [3, 29].

Finally, we would like to draw attention to some recent experimental observations which indicate that cytoskeletal elements in general might also fulfill nuclear functions. Mithieux *et al.* [78] conducting a

detailed *in vitro* study on the interaction of tubulin with histone H1 and core histones obtained results very similar to those described in the present communication. These authors detected a stoichiometric association of tubulin with the various histone species and a clear dependency of complex formation on the arginine content of the histones. In the presence of core histones, the linker histone H1 was not incorporated into the insoluble tubulin-histone polymers. On the basis of the high affinity of histones H3 and H4 for tubulin and the central role of these proteins in nucleosome assembly, Mithieux *et al.* [78] considered the possibility that tubulin might be involved in unfolding of the higher-order structure of chromatin. Moreover, Scheer *et al.* [79] reported on the inhibition of the transcription of lampbrush chromosomes but not of rRNA genes in response to microinjection of actin-binding proteins or antibodies specifically directed against actin into nuclei of living amphibian oocytes. Shutoff of transcriptional activity resulted in the appearance of extended meshworks of actin microfilaments in the vicinity of lampbrush chromosomes. These findings are corroborated and extended by those of Egly *et al.* [80] who have presented evidence for a possible role of actin as a transcription initiation factor for RNA polymerase B.

Whereas tubulin (for references, see [78]) and actin (for references, see [79, 80] could definitely be localized in nuclei, the situation is still ambiguous concerning the intranuclear distribution of IF proteins. Nuclear preparations subjected to various purification procedures generally turn out to contain IF proteins (reviewed in [3]). It is questionable, however, whether such material is just collapsed onto the outer surface of purified nuclei or whether it actually has an intranuclear distribution. In this respect, whole mount electron microscopic observations made by Penman *et al.* [81] and Fey *et al.* [82] on non-ionic detergent-extracted cells provide some additional information in that they show intermediate filaments contacting and perhaps entering the nuclear matrix directly underlying the nuclear envelope. In this connection, the cross-linking of IF proteins to DNA by cis-dichlorodiammineplatinum, γ-irradiation or chromium salt in Novikoff ascites hepatoma cells [83, 84] deserves special consideration.

Acknowledgements

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