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On the Variation of the Major Nuclear Envelope (Lamina) Polypeptides

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Summary

Three polypeptides which predominate in nuclear envelope and nuclear pore complex-lamina fractions are usually identified by their migration rates in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Unfortunately, their migration relative to proteins frequently used as molecular weight standards can vary significantly under different electrophoresis conditions. However, the three maintain the same order of migration. They have been separated on the basis of both isoelectric point and apparent molecular weight and characterized by their tryptic peptides. Their identity as unique proteins has been established. One appears to be a cleavage product of another while the third has regions of unique sequence. This cleavage model is supported by an *in vitro* cleavage reaction.

Introduction

Three major nuclear envelope polypeptides are located in the periphery of the interphase nucleus (1-3). These polypeptides predominate in the nuclear pore complex-lamina, a fraction of the nuclear envelope which is insoluble in nonionic detergents (4,5). In the avian erythrocyte, as compared with rat liver or HeLa cell nuclei, the smaller of the three is quantitatively a minor species (6). However, this erythrocyte polypeptide appears to increase in amount under some *in vitro* conditions (7). Studies of these proteins are hampered by the lack of specific assays for them. They have been previously identified by their migration in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). This approach is inadequate because reported molecular weights vary more widely than the differences between the individual polypeptides. A more specific identification has been achieved by two-dimensional electrophoresis, utilizing first isoelectric point separation followed by SDS-PAGE (8). In the present study, further

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characterization has been achieved by obtaining tryptic peptide maps of polypeptides purified by two-dimensional electrophoresis.

Materials and Methods

Preparation of the chicken erythrocyte nuclear envelope fraction (detergent-washed) and separation of the polypeptides by isoelectric point separation and SDS-PAGE has been described (8). Coomassie blue R-stained spots were sectioned from gels which had been stored in 10% acetic acid. Radioiodination, tryptic cleavage, and application of the samples to cellulose thin layer plates was performed as described by Elder et al. (9).

We and others have named the major nuclear envelope polypeptides on the basis of apparent molecular weight (1,2,7) but because of different determinations, the designations vary widely. For this reason and because all three polypeptides have their major concentration in the lamina region of the nucleus (see Introduction), we will refer to them by the recent designation lamin A, lamin B and lamin C (10), replacing our P75, P71 and P68 designation.

Results and Discussion

Variable electrophoretic migration. Reported molecular weights for lamin A vary from 80 000 (11) to 68 000 (12) although in a single sodium dodecyl sulfate electrophoresis system lamin A as well as lamin C from rat liver, Hela cell, and chicken erythrocyte migrate identically (6). An example of dependence on electrophoresis conditions is presented in Figure 1. In the first SDS-PAGE dimension, following Shapiro et al. (13), lamin A and lamin B migrate between transferrin and serum albumin. In the second dimension, a gradient gel with Laemli buffers (14), both migrate more rapidly than serum albumin. At the dilution of erythrocyte protein used here, lamin C is not detected. In the second dimension, it migrates with an apparent molecular weight of 63 000 (unpublished data). It is important to note that the relative migration of lamin A and lamin B monomers with respect to each other did not change because homodimers of lamin A and lamin B reverse their migration order in a single gel system (15). Thus, comparisons of the monomers between different studies, where they are identified only by relative order of SDS-PAGE migration, appear to be valid despite the fact that different molecular weight values are obtained.

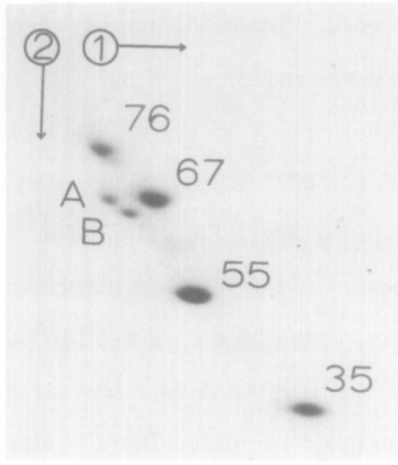

Figure 1. Mobility of lamins A and B in different electrophoresis systems. Molecular weight standards were transferrin (76 000), bovine serum albumin (67 000), glutamate dehydrogenase (55 000) and glyceraldehyde 3-phosphate dehydrogenase (35 000). The erythrocyte lamina fraction was prepared as described under Materials and Methods. Electrophoresis in the first dimension (arrow) was performed in 5% acrylamide gels in the phosphate buffer system described by Shapiro et al. (13). This gel was applied to the top of a vertical 5-20% acrylamide gel in the buffer system described by Laemli (14) and electrophoresis performed in the second dimension.

An in vitro variation in relative amounts of the lamins. With the chicken erythrocyte polypeptides, there can be an increased amount of lamin C under some conditions. An example of this increase is presented in Fig. 2. *Panel o* is the electropherogram of a predominantly lamin A fraction

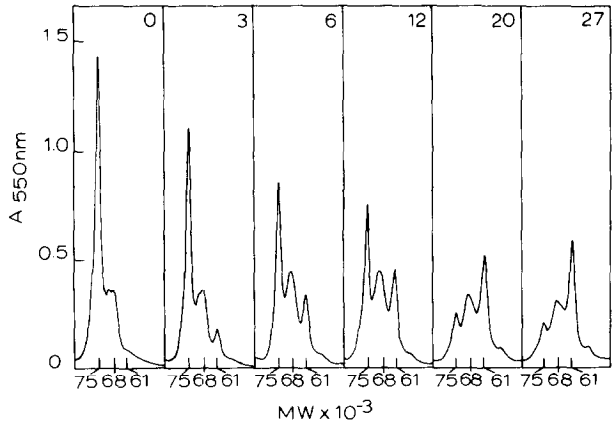

Figure 2. Formation of a 68 000-dalton polypeptide from lamin A. Lamin A was obtained by extraction from polyacrylamide gels as described previously (17). The polypeptide, in 0.10 M sodium phosphate (pH 3.5) :0.1% sodium dodecyl sulfate:1.0% 2-mercaptoethanol, was boiled for the time (min) indicated in each panel. Treated samples were analyzed on 8.5% acrylamide gel in the Shapiro et al. (13) buffers.

extracted from SDS-PAGE gels. Traces of lamins B and C are present. In the experiment, this fraction was boiled at pH 3.5 for the time (minutes) shown in each panel. The overall effect of the mild acid treatment is a significant decrease in lamin A (75 000) and an increase in a 61 000-dalton polypeptide (compare *panels 0* and *27*). There is, however, a transient increase in lamin C (68 000, *panels 6* and *12*). This experiment indicates that lamin C, as isolated from cells, might be a cleavage product of lamin A. Another explanation is that only the migration rate has varied because of a conformational change. For instance, oxidized lamin A migrates more rapidly than the reduced form, presumably as a result of a different, stable conformation (16).

The lamins are unique polypeptides. Additional support for the suggestion that lamin C is a cleavage product of lamin A derives from their respective ionic properties. In the chicken erythrocyte, lamin A appears to have only one more positive charge than lamin C and they both occur as approximately five isoelectric point variants (8). To further test that lamin C could be a cleavage product, the separate lamins were obtained from a two-dimensional gel, radiolabeled with ^{125}I, and their tryptic peptides compared (Fig. 3). Lamin C (*panel b*) yields few if any tryptic spots which differ from those found in lamin A (*panel a*). The area between the vertical lines (*panel b*) did not contain spots in a second experiment and could reflect incomplete cleavage. Lamin A yields a peptide (under the + in *panel a*) which is absent from lamin C. This could be from a fragment lost when lamin A is converted to lamin C. This evidence is consistent with lamin C being a cleavage product of lamin A but is not consistent with lamins A and C differing only by conformation. In contrast to lamins A and C, lamin B (*panel c*) yields numerous unique peptides. Lamin B differs from lamins A and C in ionic properties as well, occurring as a single isoelectric point species with a pI approximately 0.7 pH units more acidic than that of lamin A (8).

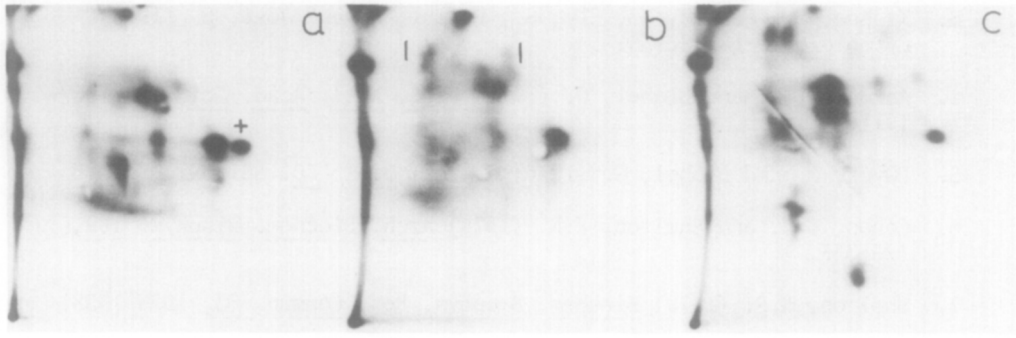

Figure 3. Radioiodinated tryptic peptides of the lamins. Samples, prepared as described under Materials and Methods, were spotted in the lower left corners. Chromatography (butanol:pyridine:acetic acid:water; 32.5:25:5:20), was performed in the first dimension. The dried plates were then moistened with electrophoresis buffer (acetic acid:formic acid:water, 15:5:80), and electrophoresis performed in the second dimension. Peptides were revealed by autoradiography. The detailed procedures have been described (9). The tryptic peptides of lamins A and C are in *panels a* and *b* while those of lamin B are in *panel c*.

The chemical relationship of the lamins. These studies permit the specific chemical identification of the lamins. They indicate a basis for the immunological cross-reaction between lamins A and C (1, and S. Ely (quoted in (3)), for the very similar topographies of lamins A and C in the HeLa matrix fraction (6) and the rat liver nuclear envelope (16), and for their similarity as detected by one-dimensional SDS-PAGE mapping (16). The relationship of lamin B to lamin A remains to be established. The evidence presented herein, topological studies (15,16), and one-dimensional SDS-PAGE maps (16) indicate different structures and functions for these polypeptides. On the other hand, imunological cross-reactivity (1, S. Ely (quoted in (3)), amino acid compositions (17) and the production of some common peptides suggest that they are related proteins (17).

Acknowledgments

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