Oligomeric Structure of the Major Nuclear Envelope Protein Lamin B*

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The nuclear envelope is a prominent and easily recognized cytological feature of eukaryotic cells. However, the molecular architecture of this complex structure is poorly understood. A promising approach to the study of nuclear envelope ultrastructure has been to determine interpolyptide contacts. This approach is appealing because the envelope fraction can be converted largely to homotypic oligomers via depolymerization, an easy step where disulfide bonds are oxidized.

In the present study, a third, smaller oligomer of lamin B has been detected. Because there are three oligomers of lamin B, it is possible that these are a dimer, a trimer, and a tetramer. Support for this model has been obtained by analysis of cross-linked fragments from a mild trypsin digestion of cross-linked lamin B proteins. This procedure yields 62,000-, 46,000-, and 30,000-dalton fragments of lamin B. Each fragment also occurs as three homotypic oligomers.

The largest oligomer, which appears to be a tetramer, is obtained in high yield under brief, mild cross-linking conditions in several cell types. These results suggest that lamin B occurs as a tetramer in the nuclear envelope.

Materials and Methods

Preparation of Nuclear Lamina Fractions from Chicken Erythrocytes and Rat Liver—Preparation of these fractions has been recently described (10). When proteins were to be cross-linked in the envelope faction, the preparations were interrupted before the Trion X-100 washing step. Cross-linking was accomplished by exposure of the envelope fraction to cupric sulfate, phenanthroline for 1 min at room temperature as previously described (4). Cross-linking reagent was then destroyed by addition of 25 μmol of EDTA and 6 μmol of 2,6-di-tert-butyl-4-hydroxymethyl phenol, and isolation of the lamina fraction continued. The final protein was dissolved in 4% SDS and frozen until used. Protein was determined by the method of Lowry et al. (11).

Tryptic Digestion under Mild Conditions—Protein was precipitated from SDS solution by combination in a 1- to 5-volume ratio with ice-cold acetone-NH₄OH (5:3:0.3) and centrifugation at 12,000 × g for 2 min in a Sorvall HB-4 rotor. After drying under a stream of nitrogen, the protein (0.5 mg/μl) was dissolved or suspended in Tris-HCl (pH 6.8), 2 μl urea. The solution was chilled in an ice bath and trypsin added as detailed in the figure legends. The samples were maintained in an ice bath overnight after which they were adjusted to 1.2 mM phenylmethylsulfonyl fluoride and 0.25% SDS to stop digestion. Glycerol (10%) was added preparatory to electrophoresis.

Electrophoresis—A variety of electrophoretic conditions were used as described under figure legends. Buffers used were the phosphate system of Shapiro et al. (12) and the Tris-glycine-SDS system of Lasnittii (13).

The abbreviations used are: SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; phosphate buffer system, running buffer (100 mM sodium phosphate (pH 7.2), 0.1% SDS)/sample buffer (10 mM sodium phosphate (pH 7.2), 0.1% SDS); Tris-glycine buffer system, upper gel buffer (125 mM Tris-HCl (pH 6.8), 0.1% SDS)/lower gel buffer (375 mM Tris-HCl (pH 8.8), 0.1% SDS)/running buffer (25 mM Tris, 192 mM glycine (pH 8.2), 0.1% SDS)/sample buffer (62.5 mM Tris-HCl (pH 6.8), 2.3% SDS).

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**Electrophoretic Analysis of Lamin B Oligomeric Structure**

125I-Tryptic Peptide Mapping—The procedure of Elder et al. (14) for analysis of stained polypeptides in gel slices was applied to lamin fragments in the two-dimensional gels.

**Molecular Weight Estimations**—Molecular weights were estimated in the discontinuous buffer, gradient polyacrylamide gels by comparison of the proteins with standards of similar molecular weight. Lamin B (66,000) and its largest cleavage product (62,000) were compared with transferrin, serum albumin, and glutamate dehydrogenase. Other lamin fragments involved in homotypic oligomers compared with transferrin, serum albumin, and glutamate dehydrogenase. Other lamin fragments involved in homotypic oligomers compared with transferrin, serum albumin, and glutamate dehydrogenase.

**RESULTS**

Anomalous Migration of Oligomeric Lamin B—It has been proposed that rat liver lamin B occurs as a trimer (7) and that chicken erythrocyte (4) and HeLa cell (9) lamin B's occur as dimers. However, this apparent difference in migration of the disulfide cross-linked oligomer may have resulted from the use of different electrophoresis conditions. Indeed, by reversing the buffer systems used in the above studies, an association between migration and buffer can be demonstrated. Cross-linked oligomers are first subjected to electrophoresis in the buffer to be tested, and then disulfide bonds are reduced and lamin A and B identified by electrophoresis in a second dimension (Fig. 1). Monomeric proteins migrate to a position on a diagonal line, while proteins which were oligomers in the first dimension yield spots below this diagonal. Oligomeric lamin B of chicken erythrocytes appears to comprise two forms migrating very near trimeric lamin A (Fig. 1a). Although this result contradicts the previous size determined for the chicken erythrocyte lamin B oligomers (4), the buffer system here is Tris-glycine, used to identify rat liver lamin B as a trimer (7). Although there is less cross-linked protein, the same results are obtained with protein presumably cross-linked by air (Fig. 1b). In contrast, when rat liver lamins are examined with the phosphate buffer system in the first dimension (previously used to identify chicken erythrocyte lamin B as a dimer (4)) lamin B oligomer migrates very near lamin A dimer (Fig. 1c). Neither system yields an entirely satisfactory answer because the largest "trimer" is 2.8-fold larger than the monomer, while the "dimer" is 2.3-fold greater than monomer in the above experiments. Thus, the oligomeric state of lamin B cannot be resolved by these experiments. Evidence for the number of monomer chains in these oligomers has been obtained from other studies.

**Limited Trypsin Digestion of Disulfide-cross-linked Lamin B under Mild Conditions**—Cleavage fragments of relatively large size can be obtained from the lamins by digestion in 2 M urea in an ice bath with low levels of trypsin (Fig. 2). These lamins had been cross-linked by disulfide bond formation in the nuclear envelope fraction. Digestion with trypsin yielded new bands both above and below the lamins monomers. These digested preparations were examined for cross-linked fragments by two-dimensional electrophoresis.

Analysis of Cross-linked, Trypsin-cleaved Lamins by Two-

**Fig. 1. Effect of electrophoresis buffer system on the migration of lamin B oligomers.** The nuclear envelope fractions were prepared as described under "Materials and Methods." Protein in the gels was stained with Coomassie Brilliant Blue. a, cross-linked chicken erythrocyte nuclear envelope protein (217 μg) was separated in the first dimension in 7% polyacrylamide gels, cross-links were broken by exposure to 2-mercaptoethanol and electrophoresis performed in the second dimension in a 5-20% gradient polyacrylamide gel. The discontinuous Tris-glycine buffer system was used in both dimensions. SDS was present in both dimensions in this and all other gels. The identification of lamin A polymers is based on their relative migration. The tetramer (A) is barely separated from material which did not enter the first dimension. Two lamin B oligomers can be detected. b, this sample (188 μg) was prepared like that presented in a except that no cupric sulfate and phenanthroline was added to promote cross-linking. c, cross-linked rat liver nuclear lamina protein (270 μg) was separated in the first dimension in the phosphate buffer system in 5% polyacrylamide gels. After reductive cleavage the proteins were separated in the second dimension as described above. In this procedure oligomeric lamin B migrates slightly more slowly than dimeric lamin A.

**Fig. 2. Mild tryptsin digestion of cross-linked erythrocyte lamina proteins.** Protein was prepared and digested as described under "Materials and Methods." The amount of trypsin in each digest is presented under each lane (percentage of total protein). Lamins A and B are indicated by dots. Molecular weight standards were ovalbumin, glyceraldehyde 3-phosphate dehydrogenase, soybean trypsin inhibitor, myoglobin, cytochrome c, and bovine liver trypsin inhibitor. Electrophoresis was in the discontinuous Tris-glycine buffer system and protein was stained with Coomassie brilliant blue.
**Electrophoretic Analysis of Lamin B Oligomeric Structure**

**Two-dimensional Electrophoresis**—The electrophoretic conditions used in this study differed from those of Fig. 1a in that electrophoresis in both dimensions was performed with the discontinuous Tris-glycine buffer system in 5–20% gradient polyacrylamide gels. 5% stacking gels were used in each dimension, and the SDS concentration was increased from 0.1–0.4% in both dimensions. The effects of these new conditions on undigested (control) protein are presented in Fig. 3a. Protein in the stacking gels was poorly resolved and is not presented. Lamin A dimer (A2) migrated as expected but trimers and larger oligomers (A3) were located at the top of the first dimension gel.

Several new features of oligomeric lamin B were revealed by these conditions. As indicated by the designations B1 and B2, two lamin B types which differ slightly in molecular weight were separated. These two proteins, which have been identified as types of lamin B by isoelectric focusing and 125I-tryptic peptide mapping have only been found in chicken cells thus far (15). As demonstrated in Fig. 3a, lamin B1, a relatively minor type, forms a predominant oligomer (B1IV) very similar to that formed by lamin B2 (here indicated as B2IV). Because it is difficult to distinguish B1 and B2 by tryptic mapping and because they yield very similar disulfide cross-linked oligomers, in the remainder of this paper we will assume that they behave as a single protein unless otherwise noted.

Another feature of this electrophoresis system is that three different oligomers of lamin B can be detected, in contrast to the two oligomers apparent in Fig. 1a. These oligomers are designated as B2IV and B2III, corresponding to the two oligomers indicated as B, in Fig. 1a, and as B2II, an oligomer which has not been detected previously.

Examination of cross-linked lamina protein in this electro-phoresis after mild trypsin digestion revealed numerous peptides from cross-linked fragments (Fig. 3b). In general, two types of oligomers can be distinguished by two-dimensional electrophoresis. For heterotypic oligomers, two or more polypeptides are observed on a single vertical line in the gel region below the "monomer" diagonal line. For homotypic oligomers, only a single polypeptide spot will occur. Examination of the spots arising from cross-linked proteins in Fig. 3b indicates that many spots arise from homotypic oligomers.

This pattern was unexpected for trypsin-digested material because it indicates that for each digested oligomeric molecule, each polypeptide chain is digested to the same extent. A series of different homotypic oligomers of different molecular weights but having the same number of polypeptide chains, for instance dimers, will yield a series of spots along a line in analogy with the diagonal streak of monomers described in Fig. 1. There appear to be three such lines of spots arising from oligomers in Fig. 3. These are identified as II–IV (I indicates monomers). These lines presumably arise from dimers (II), trimers (III), and tetramers (IV), although this is not necessarily true (see "Discussion"). Most of the spots in Fig. 3b occur on one or another of these three lines.

The spots from cross-linked oligomers which fall on the three diagonal lines also occur on a group of horizontal lines. (These lines are identified by two digits indicating the molecular weight in thousands of a protein migrating to that point in the second dimension.) Location on a common horizontal line suggests the identity of the polypeptides. This possibility was tested as follows.

**125I-Tryptic Peptide Maps of Cross-linked Proteins**—The protein spots along horizontal lines was subjected to 125I-tryptic peptide mapping in order to determine if a single protein was found in each line of spots. In this method certain amino acid residues (principally tyrosine (16)) are 125I-labeled, the protein is subjected to complete tryptic digestion, peptide maps are prepared, and the 125I-labeled peptides are detected by autoradiography. The advantages and the limitations of this method have been discussed previously (17). The principal advantage is in the comparison of sequence similarities for the trace amounts of protein purified by analytical electrophoresis. The principal disadvantages are that not all peptides will be labeled and detected, and for large proteins it may be difficult to resolve all spots. In relation to the last point, complications arise because spots increase significantly in size as sensitivity is increased by longer exposure times and because there can be a wide range in the extent to which spots are labeled.

When the protein in the spots arising from cross-linked fragments was subjected to 125I-tryptic peptide mapping, the results indicated that spots along each horizontal line contained only one protein. Autoradiograms for the 46,000- and 30,000-dalton proteins are presented in Fig. 4. Within each molecular weight class the maps for the three spots are

![Fig. 3. Two-dimensional electrophoresis of erythrocyte lamina proteins after mild trypsin digestion. Separation in both dimensions was performed with the discontinuous Tris-glycine buffer system, 5–20% gradient polyacrylamide gels, and 5% acrylamide stacking gels. The lamina protein was prepared from chicken erythrocytes as described under "Materials and Methods." Disulfide bonds were reduced after electrophoresis in the first dimension as described under Fig. 1. a, control, undigested chicken erythrocyte lamina proteins (350 μg). B1, B2, two types of lamin B differing slightly in molecular weight. II, III, IV, oligomers of lamin B. b, lamina protein (375 μg) digested with 0.375 μg of trypsin as described under Fig. 2. I, the line on which monomers are located. II, III, IV, lines on which dimers, trimers, and tetramers appear to fall. 66, 62, 46, 30, proteins of these molecular weights (in thousands) would be located on these horizontal lines. Proteins were stained with Coomassie brilliant blue.](http://www.jbc.org/content/255/11/4330)

![Fig. 4. 125I-labeled tryptic peptides. Polypeptides from gels such as those presented in Fig. 3b were "mapped" as described under "Materials and Methods." Peptides were detected by autoradiography. Panels are labeled by content.](http://www.jbc.org/content/255/11/4330)

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virtually identical. However, as discussed above, these maps cannot prove complete identity because some peptides may not be labeled and some labeled peptides may not be resolved.

In this regard, the proteins identified as 46IV, 46III, and 46II each includes two barely separated polypeptides (see Fig. 3b). These polypeptides have been separately mapped but these maps were not distinguishable. It is not known whether these closely related proteins, which differ slightly in molecular weight, reflect differences in lamin B1 and lamin B2 (it will be seen that the 46,000-dalton protein is a cleavage product of lamin B), or whether they reflect two alternative tryptic cleavage sites that are relatively close together. It is noteworthy, however, that each oligomer (II, III, and IV) contained both types. Thus, although the 125I-tryptic maps do not prove that the proteins in each oligomer were identical, the evidence of the maps plus the common molecular weights provide a strong indication that two closely related approximately 46,000-dalton proteins each occurs in three oligomeric forms.

The spot at 30II has been resolved into two spots of molecular weights 30,000 and 27,000. Again, these were not distinguishable by 125I-tryptic mapping. However, only the 30,000-dalton protein occurred in three oligomeric forms. Further, the two proteins are closely related in sequence. The 62,000-dalton protein appears to be a tryptic cleavage product of the 66,000-dalton protein. The missing 4,000-dalton fragment(s) were not detected in the 125I-tryptic peptide maps either because they were not labeled or because they co-migrated with 125I-labeled peptides retained in the 62,000-dalton protein.

The proteins identified as 66IV and 66III appeared to be the same two lamin B oligomers indicated as BIV in Fig. 1a. In order to confirm this identification, a sample of digested lamina protein was completely reduced and examined by two-dimensional electrophoresis (pI versus M;). Some undigested lamin B was found as well as a cleavage product of slightly lower molecular weight with a very similar pI (results not shown). The proteins at 62IV and 62III (Fig. 3b) which are analogous, in relative amount and separation, to the spots at 66IV and 66III reflect the slightly smaller lamin B fragment. The 125I-tryptic peptide map of lamin B, purified by isoelectric point and molecular weight, also indicated that the 66,000-dalton protein was lamin B (Fig. 5).

DISCUSSION

Several features of the lamins limit the available methods for characterizing their molecular contacts in the nuclear envelope. Of foremost importance is their isolation, together with a number of less abundant proteins, in an insoluble fraction. It has not been possible to purify native lamins from interphase nuclei; indeed, no methods are available to assess denaturation of these proteins.

Protein cross-linking reactions provide a method for identifying contiguous proteins in organized structures (6). The major advantage in this approach is that cross-linking probes are introduced before the lamins are dissolved and thereafter denaturing conditions can be used to separate and analyze the lamins. These denaturing conditions ensure that other, initially associated insoluble proteins do not affect electrophoretic analysis of the cross-linked proteins.

Results obtained indicate that the disulfide cross-linked lamin oligomers reflect native interactions. The most important finding is that, although lamins A and B are both abundant in the nuclear envelope and each reacts extensively under brief, mild reaction conditions to form homotypic oligomers, a variety of heterotypic oligomers is not obtained as would be expected from randomly aggregated molecules (4, 5). Further, the same products are obtained in low yield by air oxidation as are obtained in high yield when disulfide bond formation is promoted by treatment with cupric sulfate, o-phenanthroline, and the same products are obtained from rat liver and from chicken erythrocyte lamins. These results uniformly indicate that lamins A and B each have a highly restricted topography in the envelope, a finding consistent with their relative insolubility.

It is likely that there are lamin interactions which have not been revealed by disulfide bond formation. Otherwise, it would be difficult to explain the co-isolation of the lamins plus other proteins in the lamina fraction which is almost entirely protein (18). The absence of other disulfide-bonded oligomers most likely reflects the absence of appropriate sulfhydryls at contact sites. This absence of more extensive cross-linking is an advantage in the present study because very large products are

![Fig. 5. 125I-Labeled tryptic peptides of 62IV, 66III, 66II, and lamin B. The conditions are described in the legend to Fig. 4. Panels are labeled by content. Lamin B was purified by isoelectric point and molecular weight.](image-url)
Electrophoretic Analysis of Lamin B Oligomeric Structure

Lamins A and B are distinguished from each other both by the oligomeric products they yield and by the resistance of their cross-linked products to tryptic digestion. For example, under the conditions of these experiments, the yield of lamin A oligomers decreases as the size increases, but the largest lamin B oligomer is obtained in greatest yield. Further, lamin A oligomers appear to migrate in SDS-polyacrylamide gel electrophoresis as expected for typical proteins (12), but the lamin B oligomers do not. Finally, under the tryptic digestion conditions (phosphate-buffered, 2 mM urea), cross-linked lamin A disappears and no cross-linked fragments have been recovered. A prominent 46,000-dalton lamin A monoclonic fragment has been identified by tryptic mapping (results not shown). Thus it appears that the lamin A interpolyptide disulfide bonds are released in fragments too small for detection in 5–20% gradient polyacrylamide gels.

A surprising result of the mild tryptic digestion was the preponderance of homotypic oligomers among the fragments of lamin B. It appears that each monomer in a lamin B oligomer is cleaved at the same site in an all or none fashion. The reason for this phenomenon is unknown.

There may be some variation in the location of interpolyptide disulfide bonds in the lamin B oligomers. Such a variation could explain why there is more 30II than 30III or 30IV, and more 46II than 46IV, although there is considerably less 4611 than 46111 or 46112 (Fig. 3). Part of this shift in relative amount from higher to lower oligomers, a shift which occurs when the molecules are truncated, could be due to the higher oligomers having a greater susceptibility to proteolysis. However, there appears to be insufficient 66II to yield the observed 30II (compare a and b in Fig. 3). This increased dimer could be explained by a variation in disulfide bond location as depicted in Fig. 7.

The number of protein chains in the lamin B oligomers has been difficult to determine. In earlier studies two oligomeric forms of lamin B were detected. In one study the two chicken erythrocyte oligomers migrated as expected for dimers and they were judged to be two forms of dimer, each stabilized by disulfide bonds (4). The rat liver oligomers migrated as expected for trimers and were assumed to be two forms of trimer (7). The different results obtained for chicken and rat proteins were ascribed to inherent differences in the proteins (7). Comparison of the electrophoretic systems, however, indicates that the oligomers from the two species are very similar. Further, the detection of three lamin B oligomers suggests other possible models. These are three conformations of dimer, three conformations of trimer, a dimer and two conformations of trimer, or a dimer, trimer, and tetramer.

Molecular weight estimates based on SDS-polyacrylamide gel electrophoresis are not sufficient to determine the number of chains in the lamin B oligomers because of the dependence of migration on conditions. Some cross-linked proteins are known to exhibit anomalous migration, presumably because SDS binding is inhibited and/or the protein-SDS complex cannot assume a typical conformation (19). Apparently the binding of SDS or the conformation of the lamin B oligomer-SDS complexes varies sufficiently under the different conditions to affect their migration.

The dimer-two trimers and the dimer-trimer-tetramer models appear to be more likely than the first two models because they each predict one dimer and at least one trimer, thus minimizing the number of conformations to be stabilized.

The dimer-two trimer model is the one most consistent with the Tris-glycine buffer results if only apparent molecular weights in a single system are considered (see Fig. 1a). However, with the apparent molecular weights in doubt because of the observed variability, the dimer-trimer-tetramer model is appealing because of the cross-linked fragments obtained from lamin B. The 62,000-, 46,000- and 30,000-dalton fragments were each obtained as three oligomers. The dimer-two trimer model would require covalent maintenance of three chains and two conformations after removal of more than half of each chain. In contrast, the dimer-trimer-tetramer model would require bonds between four chains but would not require maintenance of two electrophoretically separable conformations in the various truncated molecules. The data support a tetramer model for lamin B in the nuclear envelope. The association of this tetramer with other nuclear envelope constituents remains to be determined.

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