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SELECTIVE EFFECTS OF NONIONIC DETERGENT AND SALT SOLUTIONS IN DISSOLVING NUCLEAR ENVELOPE PROTEIN

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SUMMARY

Protein has been selectively extracted from isolated chicken erythrocyte nuclear envelope by (1) dilute $MgCl_2$/Triton X-100 followed by (2) concentrated $MgCl_2$/Triton X-100 solutions. Certain proteins appear to be selectively dissolved in the first solvent and may occur in the nuclear envelope primarily as lipoproteins. Among the proteins insoluble in the low $MgCl_2$/Triton X-100 wash, as well as in 500 mM $MgCl_2$ without Triton previously used in the preparation of the envelope fraction, the quantitatively major polypeptides dissolve in a combination of high $MgCl_2$ and Triton X-100. Further, much of this dissolved protein precipitates when the $MgCl_2$ concentration is lowered by dialysis. The insolubility of these proteins appears to result from a combination of ionic and hydrophobic interactions and may explain the resistance of nuclei to various manipulative procedures including nonionic detergent washes. The procedures described provide a route for gently and selectively dissolving representative proteins from the nuclear envelope lipoprotein matrix and from the envelope "residual" protein.

INTRODUCTION

Triton X-100 has frequently been used to dissolve membrane proteins without denaturation [1–3]. We have recently described the isolation and characterization of the chicken erythrocyte nuclear envelope fraction [4]. Here we have investigated the soluble and insoluble protein resulting from Triton X-100 extraction of the isolated envelope.

Triton X-100 has found a special use in nuclear studies as a reagent which frees nuclei of adherent cytoplasmic material. In a germinal paper Blobel and Potter observed that it was ". . .effective in removing the outer nuclear membrane without further disruption of the nuclei." [5]. Despite the wide use of detergent washes in nuclear isolations, however, the precise response of nuclei is not known. Although early reports with other mild detergents [6] and with Triton X-100 [7] supported the view that the outer nuclear membrane was selectively removed, more recent studies have indicated that the detergent effect is more extensive and that both nuclear

membranes are removed [8, 9]. Efforts to describe residual nuclear morphology have also yielded variable results. The retention of nuclear morphology after a mild detergent wash has been variously ascribed to either the inner nuclear membrane [6], a particular form of chromatin termed "perinuclear chromatin" [9], or an insoluble proteinaceous material which may correspond to a pore complex-peripheral lamina [8] or a nuclear matrix [10].

In contrast to work with isolated nuclei, our approach has been to investigate solubility classes of protein in the isolated nuclear envelope. This approach permits the use of conditions that would lead to problems with dissolved deoxyribonucleohistone if applied to nuclei.

METHODS

The preparation of the nuclear envelope fraction has been described previously [4]. In this method saline washed erythrocytes from mature chickens are homogenized by nitrogen cavitation. The nuclei, in saline/1.0 mM $CaCl_2$, are collected by low speed centrifugation through a barrier of 20 % glycerol in the same buffer. The nuclei are further washed by three cycles of suspension in and low speed centrifugation from 250 mM sucrose/50 mM Tris · HCl (pH 7.5)/25 mM KCl/5 mM $MgCl_2$ (nuclear buffer with sucrose). The washed nuclei are suspended in 25 ml nuclear buffer per original ml packed erythrocytes and digested with 10 μg pancreatic DNAase/ml for 10 min in an ice water bath. A weight of solid potassium citrate equal to 10 % of the suspension volume is added slowly with stirring. Crude nuclear envelope is collected by centrifugation for 30 min at 220 000 × g. This fraction is digested overnight in one-half to one-fifth of the previous volume of nuclear buffer containing DNAase. An equal volume of cold 1.0 M $MgCl_2$ is added slowly with stirring and the nuclear envelope fraction collected by centrifugation for 40 min at 35 000 × g. The nuclear envelope is freed of excess $MgCl_2$ by suspension in nuclear buffer with sucrose and centrifugation for 20 min at 35 000 × g. This nuclear envelope fraction is composed of approximately 66 % protein, 29 % phospholipid, 3 % RNA, and 1 % DNA [4]. It is relatively free of plasma membranes on the basis of ultramicroscopic examination of the nuclear preparation and gel electrophoretic comparison of nuclear envelope and plasma membrane polypeptides [4]. Its freedom from chromatin is indicated by ultramicroscopic examination and low DNA content [4] as well as the near absence of histone (this paper).

The preparation of histone and globin has been described previously [4]. Residual nuclear protein was prepared from nuclear buffer with sucrose/0.50 % (w/v) Triton X-100 washed, twice 0.4 N H_2SO_4 extracted nuclei as described previously [4] with the exception that insoluble material was collected by centrifugation after DNAase treatment without the addition of perchloric acid. Partially purified histone fractions were prepared by a combination of solvent and acid extractions as described by Sanders and McCarty [11]. Crude histones F1 and F2c were prepared by 5 % trichloroacetic acid extraction of Triton washed chicken erythrocyte nuclei [12]. These fractions were used to tentatively identify histone bands after electrophoresis in 8 M urea/0.9 N acetic acid/0.5 % Triton X-100.

Protein was measured by the method of Lowry et al. [13]. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate followed our previous

method [4]. Triton containing gels were identical with our previously described urea-acetic acid gels [4] except that the sample buffer and gels contained 8 M urea/0.9 N acetic acid/0.5 % Triton X-100.

In the survey of various $MgCl_2$ and Triton X-100 combinations (Table I, Figs. 1 and 2), dissolved fractions were dialyzed overnight at room temperature against 222 volumes of 0.2 % sodium dodecyl sulfate. Samples (40 μl) were removed for protein assay and concentrated reagents were added to the remainder to constitute electrophoresis sample buffer. Insoluble pellets were dissolved in 2 % sodium dodecyl sulfate by 3 min of boiling. Samples (40 μl) were removed for protein assay and reagents added to dilute the sodium dodecyl sulfate and otherwise constitute electrophoresis sample buffer. All samples were boiled for 2–3 min in the presence of both sodium dodecyl sulfate and 2-mercaptoethanol before electrophoresis.

For isolated fractions (Table II, Fig. 3) soluble proteins were precipitated with 20 % trichloroacetic acid. Both insoluble fraction pellets and trichloroacetic acid pellets were washed with ether/ethanol (3 : 2, v/v), then suspended in 0.10 M Tris · HCl (pH 7.5)/2 % sodium dodecyl sulfate by homogenization and 2–3 min incubation in a boiling water bath. When samples did not dissolve with this treatment one entire preparation would be dissolved in Reagent C according to Lowry et al. for protein determination and another entire preparation diluted and boiled 3 min in electrophoresis sample buffer, including 2-mercaptoethanol, for electrophoretic analysis.

Protein determinations for samples dissolved in 8 M urea/0.9 N acetic acid/0.5 % Triton X-100 were performed on 20 μl samples in a total assay volume of 6.6 ml and blanks included 20 μl of solvent.

RESULTS

Our interest in the combined effects of high $MgCl_2$ and Triton X-100 on the isolated nuclear envelope was stimulated by difficulties in reproducing the qualitative

TABLE I

SOLUBILITY OF NUCLEAR ENVELOPE PROTEIN

Nuclear envelope from 1.0 ml chicken erythrocytes was incubated in 1.0 ml of 250 mM sucrose/50 mM Tris · HCl (pH 7.5)/25 mM KCl and either 0, 5, or 500 mM $MgCl_2$, to which was added either 0, 0.05, or 0.20 ml of 5 % (w/v) Triton X-100, for 20 min on ice before separation of soluble and insoluble protein by centrifugation for 20 min at 27 000 × *g*. Protein recovery data (expressed as mg/ml packed cells) are averages of duplicate determinations of one experiment.

Test solvent		Protein recovered		
$MgCl_2$ (mM)	Triton X-100 (% w/v)	Soluble	Insoluble	Soluble %
0	0.00	0.03	1.26	2
0	0.24	0.28	0.86	24
0	0.83	0.37	0.75	33
5.0	0.00	0.06	1.37	4
4.8	0.24	0.28	1.11	20
4.2	0.83	0.46	0.67	41
500	0.00	0.11	1.08	10
480	0.24	0.64	0.51	56
417	0.83	0.80	0.25	76

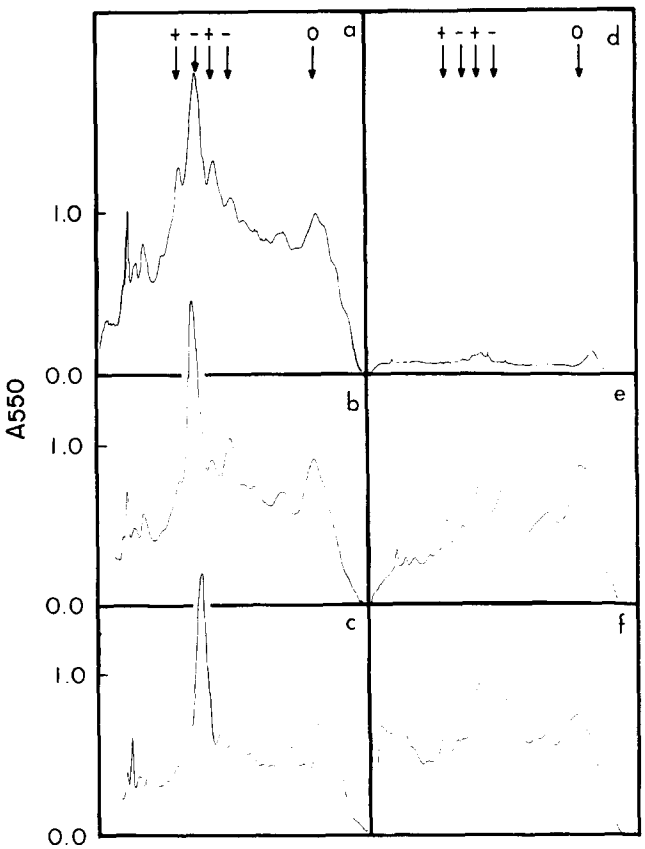

Fig. 1. Polypeptides extracted from chicken erythrocyte nuclear envelope by increasing Triton X-100 in the presence of low $MgCl_2$. Nuclear envelope was prepared as described in Methods and extracted as described in the legend to Table I (5.0–4.2 mM $MgCl_2$). Polypeptides shown in each panel derive from the following volumes of packed erythrocytes (ml): (a–c) 0.075 each, (d) 0.16, (e) 0.15 and (f) 0.13. (a), (b), and (c) represent insoluble material after extraction with 0, 0.24, and 0.83 % Triton X-100, respectively. (d), (e), and (f) represent the corresponding soluble fractions. Arrows headed by (+) indicate protein selectively soluble in Triton, (−), selectively insoluble and (0) indicates a broad band apparently containing both soluble and insoluble protein. Electrophoresis was from left to right. The gels were stained with Coomassie blue.

effects of Triton extraction. Preliminary experiments revealed that the selectivity of protein extraction was primarily dependent on whether or not the nuclear envelope pelleted from 125 mM sucrose/25 mM Tris · HCl (pH 7.5)/12.5 mM KCl/500 mM $MgCl_2$, was washed in nuclear buffer before exposure to Triton X-100. To test these observations samples from a single preparation of nuclear envelope were washed of excess $MgCl_2$ and exposed to various combinations of $MgCl_2$ and Triton X-100. The relative quantitative solvent effects are revealed in Table I. At all $MgCl_2$ concentrations, increasing Triton X-100 in the range shown increased the per cent of dissolved protein. Either 0 or 5.0–4.2 mM $MgCl_2$ in the solvent yielded similar effects. However, 500–417 mM $MgCl_2$ markedly increased protein solubility in the presence of Triton X-100. Qualitative analysis of the various soluble and insoluble fractions by

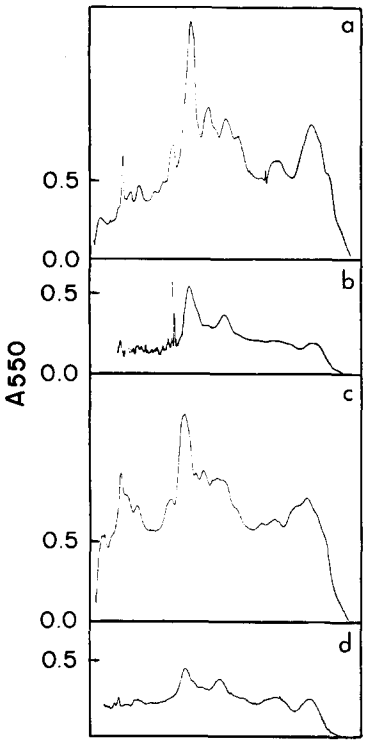

Fig. 2. Polypeptides extracted from chicken erythrocyte nuclear envelope by increasing Triton X-100 in the presence of high $MgCl_2$. Experimental details are presented in the legends to Table I and Fig. 1. Volume of cells (ml) represented in each panel is (a) 0.11, (b) 0.075, (c) 0.10, and (d) 0.075. (a) soluble in 480 mM $MgCl_2$ and 0.24 % Triton, (b) insoluble in the same solvent, (c) soluble in 417 mM $MgCl_2$ and 0.83 % Triton, and (d) insoluble in the same solvent.

TABLE II

FRACTIONATION OF NUCLEAR ENVELOPE PROTEIN

Nuclear envelope from 2.0–3.75 ml erythrocytes was extracted with 1.2 cell volumes of 207 mM sucrose/42 mM Tris · HCl (pH 7.5)/21 mM KCl/4.2 mM $MgCl_2$/0.83 % (w/v) Triton X-100 for one hour on ice. Insoluble protein here and later was collected by centrifugation for 20 min at 34 800 $\times g$. The insoluble protein was extracted with 1.2 volumes 104 mM sucrose/21 mM Tris · HCl (pH 7.5)/13 mM KCl/415 mM $MgCl_2$/0.83 % Triton X-100 for 4 h on ice. The high $MgCl_2$/Triton soluble protein was dialyzed overnight against 134 volumes of 50 mM Tris · HCl (pH 7.5)/25 mM KCl/0.5 % (w/v) Triton X-100 at 4 °C. In some experiments the protein precipitated by dialysis was dissolved and reprecipitated (total number of precipitations shown in brackets). Protein recovery data (expressed as mg/ml cells) are averages for the number of preparations (shown in parenthesis).

Protein fraction	Protein recovery
Low $MgCl_2$/Triton soluble	0.56 (4)
Low $MgCl_2$/Triton insoluble	
High $MgCl_2$/Triton soluble after dialysis	
soluble	0.27 (2)
insoluble (1 ×)	0.34 (2)
insoluble (2 ×)	0.29 (1)
insoluble (3 ×)	0.20 (1)
High $MgCl_2$/Triton insoluble	0.53 (3)

sodium dodecyl sulfate-polyacrylamide gel electrophoresis are presented in Figs. 1 and 2. Fig. 1 represents polypeptide solubility behavior in 5.0–4.2 mM $MgCl_2$. Similar results were obtained with 0 $MgCl_2$ and are not shown. Certain bands (+arrows) decrease with an increase in Triton X-100 (panels a–c) and show a concomitant increase in the soluble fractions (panels d–f). Other bands resist Triton and show an enhancement in the insoluble fractions (−arrows, panels a–c). The large band of low molecular weight protein (0 arrow) shows mixed behavior which may reflect heterogenous protein. Note that the tracing reveals several "shoulders".

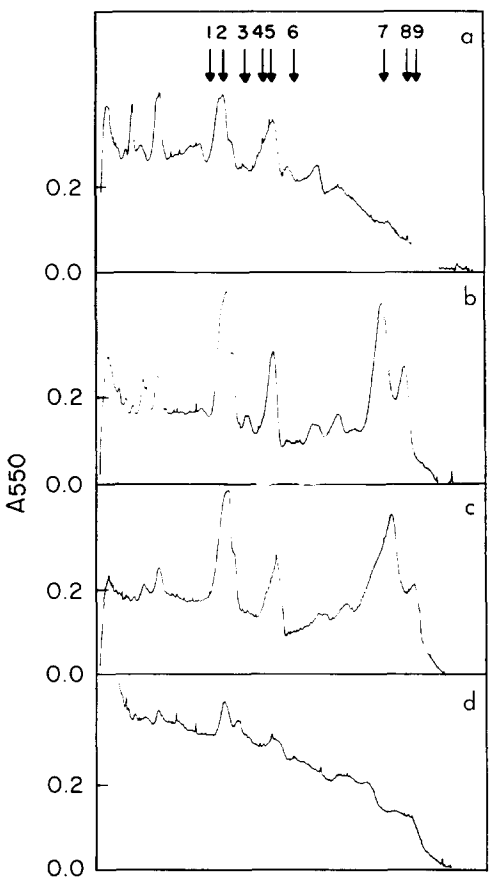

Fig. 3. Fractionation of nuclear envelope polypeptides by solubilization in high $MgCl_2$/Triton X-100 and precipitation from low $MgCl_2$/Triton X-100. Nuclear envelope was prepared as described in Methods. The polypeptides were then fractionated as described in the legend to Table II. Gels were stained with Coomassie blue. Polypeptides dissolving in the initial low $MgCl_2$/Triton X-100 wash are not shown. Polypeptides applied to each gel are quantitated as equivalent cell volumes (ml). (a) 0.144, did not precipitate upon dialysis; (b) 0.144, polypeptides precipitated by dialysis; (c) 0.144, polypeptides precipitated by dialysis were dissolved and reprecipitated by a second dialysis; and (d) 0.072, polypeptides that failed to dissolve in high $MgCl_2$/Triton X-100 in this protocol. Arrows indicate either the electrophoretic position of marker proteins: 1, phosphorylase *a*, 94 000 mol. wt.; 3, human serum albumin, 66 000 mol. wt.; 4, catalase, 60 000 mol. wt.; 6, ovalbumin, 43 000 mol. wt.; and 9, cytochrome *c*, 11 700 mol. wt. or of particular envelope bands: 2, 5, 7 and 8. Values for 1, 4, 6 and 9 are from ref. 21 and 3 is from ref. 22.

In contrast to these results, Triton extraction in the presence of 480–417 mM $MgCl_2$ (Fig. 2) significantly dissolves each of the prominent bands. Indeed, the insoluble protein (panels b and d) cannot be readily distinguished qualitatively from the soluble protein (panels a and c).

These results suggested that high $MgCl_2$/Triton X-100 might be a useful solvent for dissolving nuclear envelope polypeptides. As shown in Table II, sequential extraction first by low $MgCl_2$/Triton X-100, then high $MgCl_2$/Triton X-100 separates the nuclear envelope protein into three approximately equal fractions, quantitatively. The high $MgCl_2$/Triton X-100 soluble fraction can be further separated by dialysis to lower the $MgCl_2$ concentration. The precipitated protein is enriched for several bands identified as Triton insoluble (Fig. 3, arrows 2, 5, 7 and 8; compare with Fig. 1, —arrows).

In order to test the reversibility of the solubilization and precipitation this step was repeated once (one experiment) and twice (one experiment). Comparison of twice precipitated (Fig. 3, panel c) with once precipitated protein (Fig. 3, panel b) indicates that the operations can be repeated. However, the electropherogram of the three times precipitated protein (not shown) resembled a mixture of twice precipitated

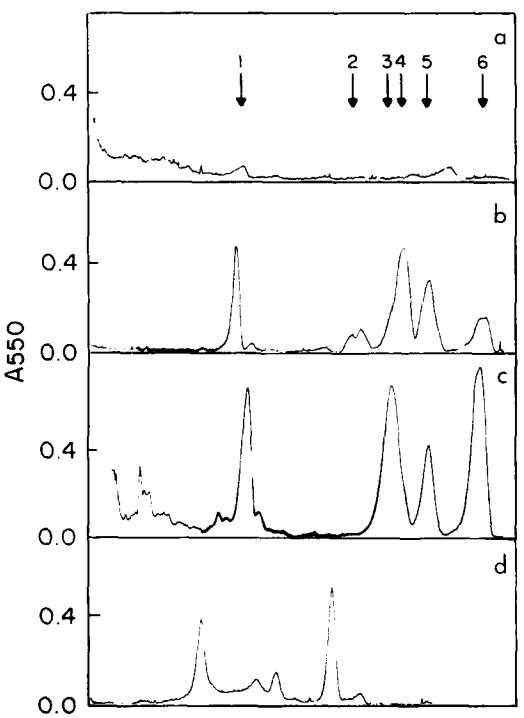

Fig. 4. Globin and histone identification by electrophoresis in 8 M urea/0.9 N acetic acid/0.5 % Triton X-100. Proteins were prepared as described in Methods. Each sample was dissolved or suspended in the above solvent and separated in 15 % polyacrylamide gels cast in the same solvent. The gels were stained with Buffalo Black. Panel (a) 138 μg nuclear envelope protein, (b) 24 μg histone, (c) 168 μg acid-washed nuclear residual protein, and (d) 28 μg hemoglobin. Arrows 1–6 indicate bands in the histone fraction tentatively identified as F2a2, F1, F3, F2c, F2b, and F2a1, respectively.

protein and protein insoluble in high $MgCl_2$ (Fig. 3, panels c and d). These results indicated that further purification of bands at arrows 2, 5, 7 and 8 is unlikely by this process and that progressive denaturation is a factor.

Hemoglobin and histone are major components of avian erythrocyte total protein and can be difficult to identify in nuclear nonhistone protein fractions from this cell [4]. In a separate study we had found that globin bound to plasma membrane and also histones associated with the residual protein of acid washed nuclei could be dissolved in 8 M urea/0.9 N acetic acid/0.5 % Triton X-100 and identified by electrophoresis in a gel system similar to that used by Alfageme et al. [14] and Gurley and Walters [15] (Shelton, K. R. and White, C., unpublished results). As can be seen in Fig. 4, the nuclear envelope is relatively free of both types of contaminants as judged by this method. Thus the heterogenous low molecular weight band represents non-globin, nonhistone protein.

DISCUSSION

Exposure of isolated nuclear envelope from chicken erythrocytes to Triton X-100 in the presence of low levels of $MgCl_2$ dissolves one-third to one-half of the protein. Certain polypeptides are selectively soluble as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The intrinsic proteins of the human erythrocyte plasma membrane are also selectively dissolved by Triton X-100 [1]. These proteins appear, by several criteria, to be buried in the lipid bilayer, thus the designation of intrinsic proteins [1]. In model studies, Clarke has shown that lipoproteins, including some integral membrane proteins, possess a large number of Triton X-100 binding sites [16]. Thus we may tentatively conclude that the protein dissolving in low $MgCl_2$/Triton X-100 is enriched in lipoproteins of the nuclear envelope.

Triton insoluble proteins have also been previously recognized. They appear to be involved in proteinaceous complexes. Yu et al. [1] have shown that several membrane bound extrinsic proteins resist Triton solubilization. Among these, spectrin, perhaps in conjunction with actin, is thought to form a continuous fibrous network providing structural support for the erythrocyte plasma membrane [1]. Triton X-100 has also been used to strip contaminating membranes from cardiac myofibrils [2], cytoplasmic membranes from bacterial cell walls [17], and as previously noted, cytoplasmic membranes and perhaps some nuclear membranes from nuclei. Aaronson and Blobel [8] have studied the effects of Triton extraction on rat liver nuclear ghosts and have concluded that the insoluble protein includes the nucleopore complex-peripheral lamina. Interestingly, their major Triton insoluble nuclear ghost polypeptides were in the 60 000–70 000 molecular weight class, while the major insoluble polypeptides in this study are above and below these molecular weights. (See Fig. 3, arrow 3 at 66 000 and arrow 4 at 60 000.)

An elevated $MgCl_2$ concentration permits much of the insoluble protein to dissolve in the presence of Triton X-100. The necessity for both high $MgCl_2$ and Triton X-100 in the same solvent is supported by several observations. Firstly, the residual envelope fraction has previously been washed with both high $MgCl_2$ and Triton X-100 separately, Secondly, following dialysis to low $MgCl_2$/Triton X-100, much of the protein precipitates. A second cycle of solubilization and precipitation yields very similar polypeptides in the same proportions. These results indicate that a

combination of ionic and hydrophobic interactions contributes to the insolubility of this class of nuclear envelope polypeptides. Some polypeptides occur in both the high $MgCl_2$/Triton X-100 soluble and insoluble fractions as judged by comparative electrophoresis. The basis of this differential extraction is not known. Although it may have a physiological significance, the insoluble protein may merely reflect protein denaturation. Its intractable state makes further study difficult. Results with various enzymes indicate that Triton exposure need not lead to protein denaturation [1–3]. The methods described herein provide an approach for the initial fractionation of nuclear envelope proteins and may provide for their further separation as soluble, nondenatured entities.

It would be of interest to know if there is a physiological role for the relative insolubility of the proteins dissolving only in high $MgCl_2$/Triton X-100. An obvious role to suggest is that of a structural element. We have previously shown that the nuclear envelope proteins include all of the quantitatively significant nonhistone nuclear proteins in this particular cell [4]. The solubility characteristics of the high, $MgCl_2$/Triton X-100 soluble proteins and their presence as a considerable portion of the total envelope protein are consistent with their involvement in a structural, element. More directly, much of the chromatin of the erythrocyte nucleus is condensed at the nuclear envelope. The chromatin of dehistonized erythrocyte nuclei decondenses in water and recondenses at the envelope upon return to physiological saline, indicating that nonhistone proteins and the envelope must be of central importance in gross nuclear organization [18]. Comings has also provided morphological evidence for the nuclear envelope providing an organizing or stabilizing element [19, 20]. In view of these considerations it appears likely that the relatively insoluble proteins described herein derive from a proteinaceous nuclear skeleton.

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