PROTEIN–PROTEIN INTERACTION IN THE NUCLEAR ENVELOPE

David L. COCHRAN and Keith R. SHELTON
Department of Biochemistry and the MCV/VCU Cancer Center, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298, USA

Received 10 September 1976

1. Introduction

Intracellular proteinaceous networks occur and may provide skeletal support in erythrocyte plasma membranes [1] and in nuclei [2,3]. Scheer et al. have provided morphological evidence for detergent resistant '. . . threadlike intrinsic membrane components . . .' in the nuclear evelope of rat liver and of amphibian oocytes [4]. We have previously shown that much of the chicken erythrocyte nuclear envelope protein occurs in an electrophoretic band of approximately 77 000 dalton [5] and that this band is especially resistant to solubilization by detergent [6,7]. Here we report the sensitivity of the 77 000 dalton polypeptides to inter-polypeptide crosslink formation via treatment of the envelope with the bifunctional imidoester, dimethyl suberimidate, and thus, the probable existence of these polypeptides in an oligomeric form in the nuclear envelope. These observations indicate that these polypeptides may fulfill a skeletal function.

2. Materials and methods

Chicken eryhtrocyte nuclear envelope was prepared from circulating erythrocytes of mature chickens as described by Shelton et al. [5]. This preparation is free of plasma membrane and chromatin contamination and is predominantely in the form of vesicles. Chemically it is approximately 66% protein, 26% phospholipid, 4% cholesterol, 3% RNA, and 1% DNA [5]. Reaction with bifunctional imidoester was effected as follows. Purified envelope fraction was washed once in 50 mM triethanolamine–HCl (pH 7.5)/25 mM KCl/5 mM $MgCl_2$ and envelope from 1.0 ml packed eryhtrocytes was suspended in 2.0 ml of the same buffer for each test solution. Imidate ester reagent was prepared as a 50 mM solution in 200 mM triethanolamine–HCl (pH 7.5). Reduced concentrations of reagent were prepared by serial dilution in water. After five min at room temperature 1.0 ml of reagent was added to each 2.0 ml of envelope suspension and reaction allowed to proceed for 20 min at room temperature. The reaction mixture was then chilled in an ice-water bath and subsequently dialyzed overnight at 4°C against 100 vol. of distilled water. For electrophoretic examination, concentrated reagents were added to the dialyzed samples to constitute sample buffer, 10 mM triethanolamine–HCl (pH 7.5)/1.0 mM EDTA/3% 2-mercaptoethanol/1% sodium dodecyl sulfate/10% glycerol. The protein was dissolved by boiling for 2–3 min. Electrophoresis and staining followed the method of Fairbanks et al. [8] as previously modified [9]. Envelope polypeptides from 40 μl of packed erythrocytes were applied to each gel. The Coomassie Brilliant Blue stained gels were scanned with a Gilford spectrophotometer. Molecular weight standards were phosphorylase *a*, human serum albumin, catalase, ovalbumin, and cytochrome *c*.

3. Results and discussion

Isolated envelope was exposed to various concentrations of the bifunctional crosslinking agent dimethyl suberimidate which reacts selectively with amino groups [10]. Inter-polypeptide crosslinks formed by these agents reflect spatial association of

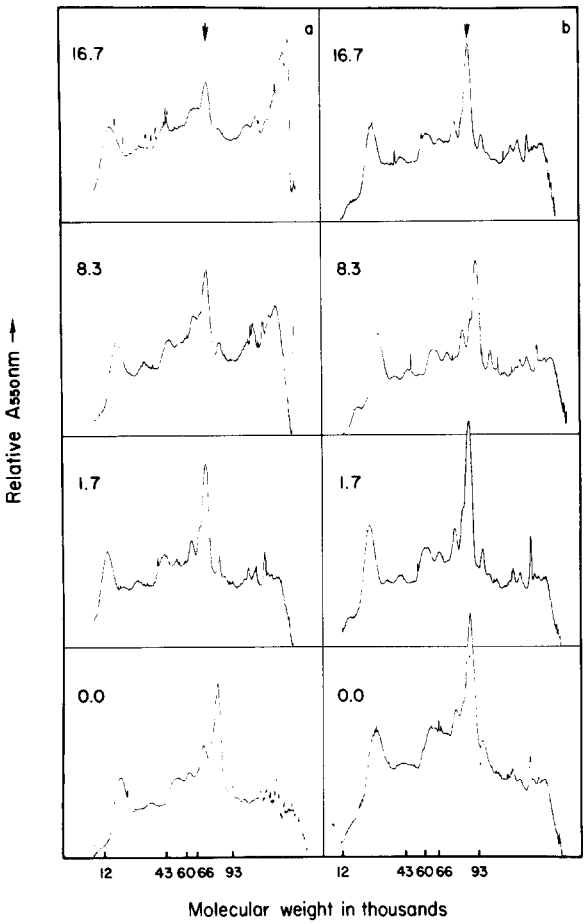

Fig.1. Electropherograms of chicken erythrocyte nuclear envelope polypeptides following exposure of the envelope fraction to various concentrations of crosslinking reagents. The two reagents used were (a) dimethyl suberimidate and (b) dimethyl adipimidate. Concentration of reagent (mM) in each test is shown in the panel with the electropherogram of the resultant polypeptides. The position of the 77 000 dalton band is indicated by an arrow.

the involved polypeptides. Reactivity of polypeptides in the nuclear envelope was assessed by dissolving the reacted envelope fraction with the aid of sodium dodecyl sulfate and thereafter examining the polypeptides by polyacrylamide gel electrophoresis. As can be seen in fig.1a, an increase in dimethylsuberimidate concentration from 0–16.7 mM caused a selective decrease in polypeptides of approximately 77 000 dalton with a concomitant increase in material greater than 200 000 dalton. At least two polypeptides can be detected in the 77 000 dalton band by electrophoresis on 4–30% gradient polyacrylamide gels [5,9] and both appear to be crosslinked (unpublished observation).

The possibility existed that the polypeptide associations indicated by these results arose randomly during the envelope isolation procedure, perhaps as a consequence of the removal of hemoglobin or of chromatin, the major erythrocyte components. If so, one would expect a range of 'reactive site distances' and thus little or no specificity with respect to similar crosslinking reagents which varied only in chain length. The chain lengths of the imidoesters vary with the number of methylene groups, therefore they provide an opportunity for assessing the distance between reactive sites [10,11]. As can be seen in fig.1b, dimethyl adipimidate at the same concentrations as dimethyl suberimidate did not specifically diminish the 77 000 dalton band or give rise to the high molecular weight material. This observation indicates that the 77 000 dalton polypeptides occur in a specific oligomeric arrangement in the isolated nuclear envelope.

We have previously shown that a variety of washing protocols applied to chicken erythrocyte nuclei yield a residue which is enriched in the 77 000 dalton polypeptides. These include preparation of nuclear envelope and 'chromatin' [5] and both Triton X-100 extraction of nuclear envelope and preparation of an apparent envelope fraction from Triton X-100 washed nuclei [6,7]. Some oligomeric enzymes resist Triton dissolution [12]. Non-ionic detergent insolubility can reflect native, non-covalent protein associations such as those found in cardiac myofibrils [13] and has been considered evidence for the physiological significance of proteinaceous structures revealed by electron microscopy in plasma membrane and nuclear residues [1–3]. The imidoester crosslinking results reported here provide independent evidence for specific nuclear envelope polypeptide associations in the presence of the lipid matrix.

Acknowledgement

This work was supported by Grant Number Ca 15923, awarded by the National Cancer Institute, DHEW.

References

[1] Yu, J., Fischman, D. A. and Steck, T. L. (1973) J. Supramol. Struct. 1, 233–248.
[2] Berezney, R. and Coffey, D. (1974) Biochem. Biophys. Res. Commun. 60, 1410–1417.
[3] Aaronson, R. P. and Blobel, G. (1975) Proc. Natl. Acad. Sci. USA 72, 1007–1011.
[4] Scheer, U., Kartenbeck, J., Trendelenburg, M., Stadler, J. and Franke, W. W. (1976) J. Cell Biol. 69, 1–18.
[5] Shelton, K. R., Cobbs, C. S., Povlishock, J. T. and Burkat, R. K. (1976) Arch. Biochem. Biophys. 174, 177–186.
[6] Shelton, K. R. (1976) Biochim. Biophys. Acta, in the press.
[7] Shelton, K. R., Lindsey, S. W., Cobbs, C. S., Povlishock, J. T. and Vandenberg, R. D. (1975) J. Cell Biol. 67, 395a.
[8] Fairbanks, G., Steck, T. L. and Wallach, D. F. H. (1971) Biochemistry 10, 2606–2617.
[9] Cobbs, C. S. and Shelton, K. R. (1975) Arch. Biochem. Biophys. 170, 468–475.
[10] Dutton, A., Adams, M. and Singer, S. J. (1966) Biochem. Biophys. Res. Commun. 23, 730–739.
[11] Davies, G. E. and Stark, G. R. (1970) Proc. Natl. Acad. Sci. USA 66, 651–656.
[12] Clarke, S. (1975) J. Biol. Chem. 250, 5459–5469.
[13] Solaro, R. J., Pang, D. C. and Briggs, N. F. (1971) Biochim. Biophys. Acta 245, 259–262.