Vol. 114, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 18, 1983 Pages 183-189

HIGH MOLECULAR MASS PHOSPHOPROTEINS IN THE RAT LIVER NUCLEAR MATRIX IDENTIFICATION OF A PROMINENT 110,000 DALTON SPECIES

Anton Schweiger and Günter Kostka

Max-Planck-Institut für Biochemie, 8033 Martinsried bei München
Bundesrepublik Deutschland
West Germany

Received May 5, 1983

SUMMARY: Purified rat liver nuclei were labelled in vitro in the presence of (32P) ATP and submitted to sequential extraction with DNAse, 0.4 or 2.0 M NaCl and Triton X-100. The residual or matrix structures contained 8-10 phosphoproteins between 76 and 260 kd including a triplet of major bands with 110, 117 and 128 kd. The 110 kd species was purified by chromatography on oligo(dT)-cellulose. It was shown to be identical with the 110 kd phosphoprotein of rat liver or Morris hepatoma free polyribosomes using the technique of limited digestion with S. aureus protease V 8.

In recent years much effort has been focussed on the investigation of a special nuclear structure, the nuclear matrix. This proteinaceous network was first observed in rat liver nuclei(1) but appears to be present in most eukaryotic cells (2-6). The matrix serves as a framework for maintaining nuclear shape and organisation, provides the structural support for specific nuclear reactions such as those involved in DNA replication (7), RNA transcription (8), processing and transport (4,9) and may bind certain low molecular weight effectors such as hormones (10,11). In addition it has been shown that matrix proteins are phosphorylated by specific protein kinases (12) and that this reaction is altered following partial hepatectomy (13) or administration of cortisol in rats (11). Several aspects of these findings indicated a possible similarity between matrix phosphoproteins and certain phosphorylated proteins from the hepatoma and rat liver polyribosomes and hnRNA-protein particles which have recently been isolated in this laboratory (14). One major species detected in all these experiments was a 110 kd phosphoprotein with high affinity for oligo(dT)-cellulose, DNA-cellulose and several ribonucleic acids. Though this protein and similar proteins were selectively bound to, and subsequently detached from oligo(dT)-cellulose together with mRNA or hnRNA we cannot at present assign them to the endogeneous mRNA-protein or hnRNA-protein complexes described in the literature (15,16). One possibility considered here is that the 110 kd protein is actually derived

0006-291X/83 $1.50

from cellular structures such as the cytoskeleton or nuclear matrix which are known to be intimately associated with free polyribosomes or hnRNA-protein particles in the cell.

In this paper we show that the nuclear matrix preparations contained tightly bound phosphoproteins in the range of 76-26o kd. 8-1o matrix phosphoproteins were detected after labelling whole nuclei in the presence of (^{32}P) ATP using aprotinin and phenylmethylsulfonyl fluoride as proteinase inhibitors. One of these proteins had a molecular weight of 11o ooo. Comparison with the corresponding protein isolated from polyribosomes showed the nuclear matrix protein to be identical with the 11o ooo species described previously.

MATERIALS AND METHODS

Beef pancreas deoxyribonuclease I (EC 3.1.4.5), purified by affinity chromatography on agarose-5'-(4-aminophenyl-phosphoryl) uridine 2'(3') phosphate, and S. aureus protease V 8 (EC 3.4.21.19) were obtained from Miles GmbH, Frankfurt, FRG. Adenosine (γ-32P) triphosphate (6-13 Ci/mmol) was obtained from New England Nuclear, Dreieich, FRG and oligo(dT)-cellulose, type 2, from Collaborative Res. Inc., Waltham, Mass., USA. Aprotinin, a polypeptide with polyvalent inhibitory action on proteinases, trade-name Trasylol, was purchased from Bayer, Leverkusen, FRG.
Nuclei and free polyribosomes were prepared from livers of 2oo g male Wistar rats as described previously (17). All buffer solutions included 14 µg aprotinin/ml and 1 mM phenylmethylsulfonyl fluoride (PMSF).

Preparation of nuclear matrix. Purified nuclei (7-1o mg DNA) from one rat liver were submitted to sequential extraction following the procedure described by Berezney (18). Nuclei were suspended in 5o ml o.25 M sucrose, 2o mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, 14 µg aprotinin/ml and incubated with 25o µg DNAse I for 1o min at 25 °C. This incubation was performed in the presence of 5o µCi (32P) ATP to label phosphoproteins. In some cases DNAse-digested nuclei were extracted with o.4 M NaCl instead of the usual 2.o M NaCl. The high ionic strength extraction was also carried out in 2 steps from o-o.4 and o.4-2.o M NaCl.

Oligo(dT)-cellulose chromatography. Rat liver polyribosomes were labelled with (32P) ATP and passed through a column of oligo(dT)-cellulose after dissociation with puromycin-o.5 M KCl, as described previously (14). Nuclear extracts obtained with o.4 or 2.o M NaCl were adjusted to o.5 M NaCl and stirred with oligo(dT)-cellulose for 1 h in the cold. The cellulose was then filled in a column and washed with 1o mM Tris-HCl, pH 7.6, 1o mM EDTA, o.25 M NaCl. Polyribosomal and nuclear proteins were eluted with successive steps of 2.o M NaCl, and 25 and 5o % formamide.

SDS polyacrylamide gel electrophoresis and autoradiography. Nuclei, matrix preparations and aliquots from the supernatants after precipitation with TCA were analysed electrophoretically as described in (14). Re-electrophoresis with S. aureus protease V 8 was also performed as described previously (14). The gels and autoradiographs were scanned at 58o nm with a Hirschmann densitometer Elscript 4oo (Hirschmann, München, FRG).

Chemical determinations. For determination of nucleic acids an aliquot of the nuclear matrix preparation was sedimented and incubated with 2.5 ml of o.5 N perchloric acid for 15 min at 7o °C. Insoluble materials were removed by centrifugation. DNA was determined in the supernatant using the diphenylamine reaction (19) and RNA by the orcinol reaction (2o). Protein was measured in another aliquot of the matrix preparation using the method of Lowry et al. (21).

RESULTS

Isolation and characterization of the nuclear matrix. Isolated rat liver nuclei were labelled by the action of endogeneous protein kinase in the presence of (^{32}P) ATP. The nuclear matrix was obtained by extracting large amounts of material, especially proteins and DNA, in a series of steps as described by Berezney (18). The residues and supernatants separated by low speed centrifugation after each step were analysed by SDS polyacrylamide gel electrophoresis. The results are shown in Fig. 1, I and II. The initial incubation with DNAse solubilized proteins with mostly low or intermediate molecular weights (Fig. 1, I g) whereas after treatment with high ionic strength buffers the supernatants contained mostly high molecular weight proteins and histones. The residual structures obtained after treatment with o.4 or 2.o M NaCl had very similar protein compositions (Fig. 1, I b and I c). Further extraction of the o.4 M NaCl washed structure (Fig. 1, I b) with 2.o M NaCl apparently caused removal of typical matrix proteins including major species (Fig. 1, I e).

The distribution of labelled phosphoproteins in the various fractions is shown in the autoradiograph in Fig. 1, II. Short-time exposure of the dried gels revealed predominantly high molecular weight phosphoproteins with major bands close to 1oo ooo which were almost completely retained in the DNAse incubated nuclei (Fig. 1, II a). Extraction with o.4 M NaCl buffer (Fig. 1, II d) removed about 7o % and further extraction with 2.o M NaCl (Fig. 1, II e) another 2o-25 % of the 2-3 major phosphoproteins. The final 2.o M NaCl matrix preparation retained 5-1o % of the label in a tightly bound form. Fig. 1, II also shows that certain phosphoproteins appear as split bands when analysed after TCA precipitation from a solubilized state.

Fig. 2 A shows the composition of the final 2.o M NaCl matrix preparation in more detail. These patterns including those for proteins in the 9o-25o kd were highly reproducible provided that the proteinase inhibitor aprotinin was added to all buffers used in the isolation of nuclei and nuclear matrix. The three matricin species and various proteins assigned to a residual RNP fraction (18) are visible amongst the stained bands. The distribution of matrix phosphoproteins is shown in Fig. 2 B. They consist of 8-1o partly faint bands with molecular weights of 76 ooo-26o ooo. Three main components with 11o ooo, 117 ooo and 128 ooo were identified as a triplet of bands in all the preparations from normal rat liver.

The nuclear matrix prepared after treatment with 2.o M NaCl as described above was embedded, sectioned and viewed under the electron microscope. It showed the morphological characteristics known from the literature, namely the presence of a peripheral layer enclosing highly condensed residual nucleoli and an extensive granular and fibrous network forming the internal matrix

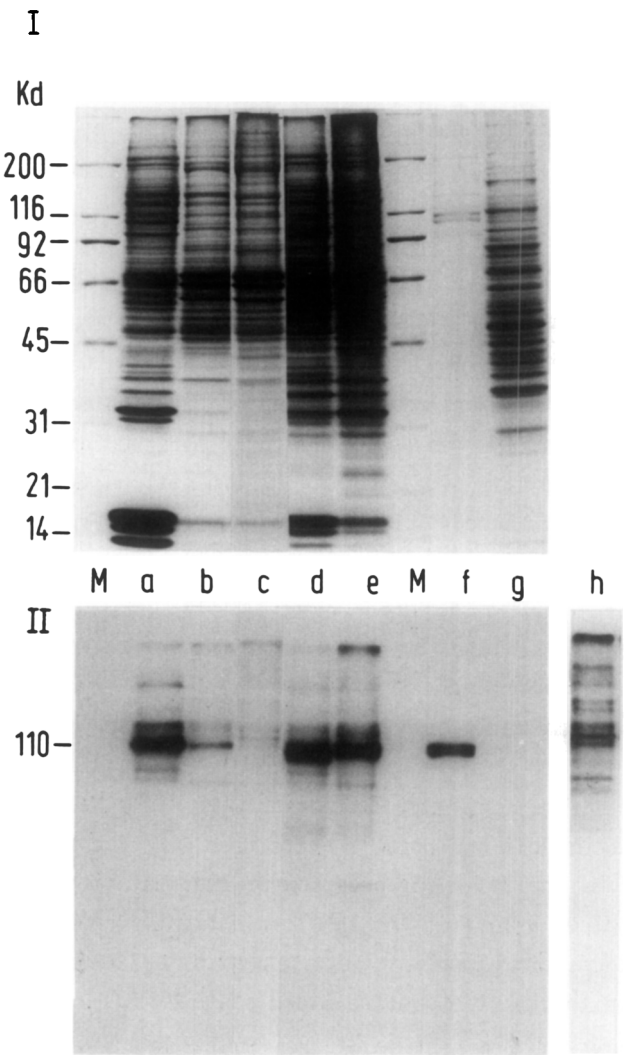

Figure 1. SDS polyacrylamide gel electrophoresis of fractions obtained in the preparation of a rat liver nuclear matrix. Liver nuclei were labelled in the presence of (32P) ATP in vitro and fractionated as described in Methods. a-c, residues after treatment of nuclei with DNAse (a), DNAse followed by o.4 M NaCl (b), DNAse followed by o.4 M and additional 2.o M NaCl (c); d, e, g, supernatants after DNAse (g), DNAse and o-o.4 M NaCl (d), DNAse and o.4-2.o M NaCl (e). f, 11o kd phosphoprotein isolated by chromatography on oligo(dT)-cellulose from supernatant separated in track e; h, phosphoproteins of the 2.o M NaCl matrix visualized by increasing the time of exposure. I, a-g, tracks stained with Coomassie blue; II, a-h, autoradiographs. M, marker proteins.

(photo not shown). The chemical composition of the preparations was also in accordance with published data (18): 79.4 ± 5.9 % protein, 17.4 ± 3.6 % RNA, 3.2 ± o.6 % DNA, each determined for 5 different samples.

Identification of a 11o kd phosphoprotein. The 11o kd phosphoprotein present at various stages in the matrix preparation was compared with a similar polyribosomal protein described previously (14). The proteins were selectively

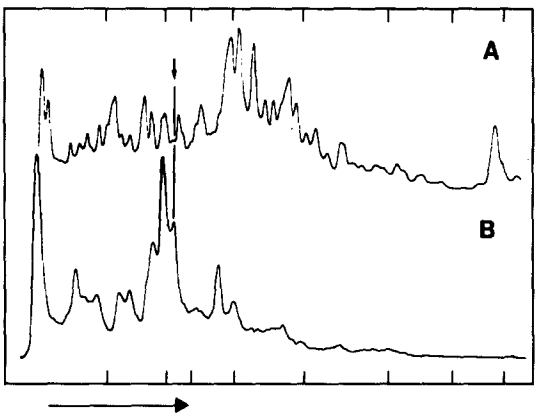

Figure 2. Densitometric profiles of nuclear matrix proteins separated by SDS polyacrylamide gel electrophoresis. The matrix was prepared (including the 2.o M NaCl extraction) as described in the legend to Fig. 1. A, bands stained with Coomassie blue; B, bands visualized by autoradiography. The arrow shows the position of the 11o kd phosphoprotein. The lines on the horizontal axis indicate the migration of the 8 marker proteins shown in Fig. 1.

bound to oligo(dT)-cellulose and detached with 2.o M NaCl (not shown). Proteins isolated from resuspended polyribosomes and from the nuclear o.4-2.o M NaCl extract were analysed by SDS polyacrylamide gel electrophoresis and autoradiography (Fig. 3). The 11o kd phosphoprotein can be seen in Fig. 1,II f after purification from the 2.o M NaCl extract shown in Fig. 1, II e. Labelled bands corresponding to the polyribosomal and nuclear 11o kd protein were cut out from both tracks and re-electrophoresed after limited digestion with S. aureus protease V 8. The results are shown in Fig. 4. Several Coomassie blue stained fragments appeared in the range of 21-45 kd and 4 of them were radioactively labelled. As a result of slightly altered conditions of protease digestion and re-electrophoresis more of the degradation products were of the smaller sizes described in the previous report (14). Fig. 4 shows that the 11o kd phosphoproteins from polyribosomes and nuclear matrix were virtually identical.

DISCUSSION

The residual or matrix structure isolated from rat liver nuclei in the present study showed the characteristic properties described in the literature for similar preparations (18). The matrix proteins included three major species, the primary matrix polypeptides or matricins, and numerous minor components spanning a wide range of molecular weights. This pattern, as well as the distribution of phosphoproteins on the gel after labelling in vitro varied as a result of the presence of proteinases. However the pattern was completely reproducible when aprotinin was added as an inhibitor to all solutions or buffers used. In one-dimensional gel electrophoresis 8-1o high

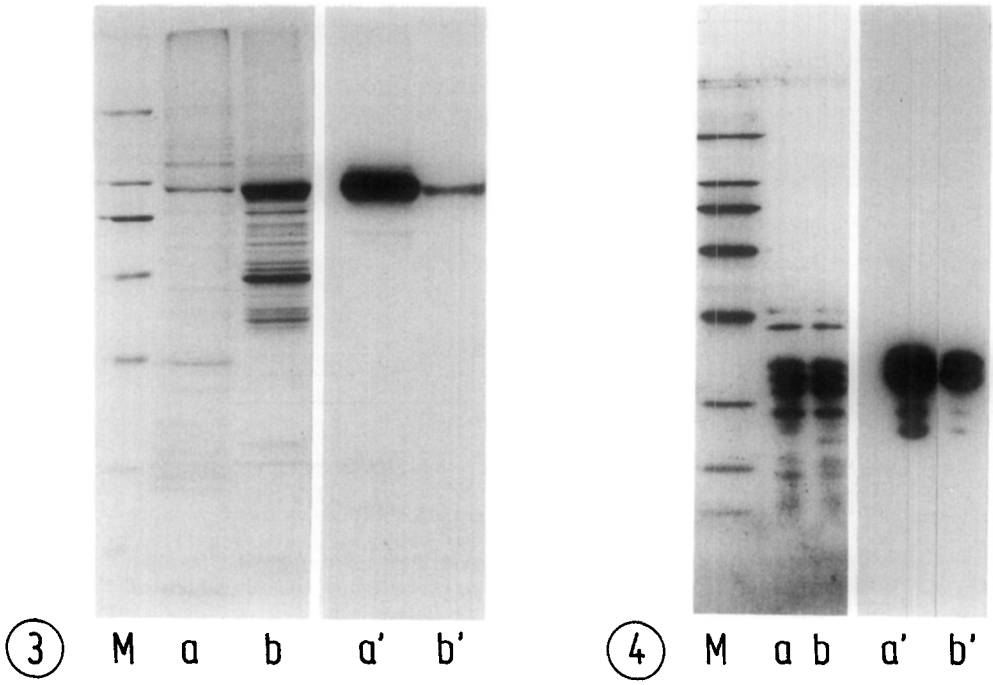

Figure 3. SDS polyacrylamide gel electrophoresis of partly purified 11o kd phosphoproteins. The proteins were labelled with (32P) ATP in vitro and eluted with 2.o M NaCl from columns of oligo(dT)-cellulose as described in Methods. a, a', proteins extracted with 2.o M NaCl from nuclear residual structure pre-extracted with o.4 M NaCl; b, b', proteins from free polyribosomes. a, b, tracks stained with Coomassie blue; a', b', autoradiographs. M, marker proteins.

Figure 4. Comparison of 11o kd phosphoproteins from nuclear matrix and polyribosomes. The 11o kd phosphoprotein shown in Fig. 3, a' and b' was cut out and re-electrophoresed following digestion with S. aureus protease V 8 as described previously (14). Tracks a and a', matrix protein; tracks b and b', polyribosomal protein. a, b, Coomassie blue staining; a', b', autoradiographs. M, marker proteins.

molecular weight phosphoproteins were detected in the normal rat nuclear matrix. Most of these proteins, however, appeared not to be restricted to the residual structure since they were identified in the whole nuclei, some in considerable amounts, being gradually detached during extractions with high ionic strength buffer. An exception was the very tightly bound 117 kd species which was retained as the main phosphoprotein in all 2.o M NaCl preparations.

In previous reports similar large size matrix phosphoproteins have been ascribed to regulative processes occurring in regenerating or cortisol-treated rat liver nuclei (12,13). Our own results have shown that treatment of rats with low doses of α-amanitin or cycloheximide causes strong and specific changes in the phosphorylation pattern of matrix proteins affecting in particular the 11o kd component (manuscript in preparation). We therefore decided to study this matrix phosphoprotein in more detail with the aim of elucidating possible functional properties. The 11o kd protein from rat liver

nuclei was (i) tightly bound to nuclear structures including the matrix, (ii) identical with a corresponding protein in rat liver free polyribosomes and (iii) identical with the 11o kd species isolated from Morris hepatoma nuclei (14).

The type of binding of specific matrix phosphoproteins is not known at present. Considering the very high affinity of the 11o kd protein for different DNAs and RNAs one may assume that this protein interacts with residual DNA fragments in the matrix or with RNA species such as newly synthesized hnRNA, or snRNAs present especially in the o.4 M NaCl matrix in considerable amounts (22). In order to investigate these possibilities we have started rebinding studies. Preliminary results indicate that there is an efficient interaction between the 11o kd phosphoprotein purified by oligo(dT)-cellulose chromatography and nuclear matrix structures prepared with 2.o M NaCl.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. E. Koch and Mrs. S. Andric for excellent technical assistance.

REFERENCES

1. Berezney, R. and Coffey, D.S. (1974) Biochem. Biophys. Res. Comm. 6o, 141o-1417.
2. Berezney, R., Basler, J., Hughes, B.B. and Kaplan, S.C. (1979) Cancer Res. 39, 3o31-3o39.
3. Long, B.H., Huang, C.-Y. and Pogo, A.O. (1979) Cell 18, 1o79-1o9o.
4. Van Eekelen, C.A. and van Venrooij, W.J. (1981) J. Cell Biol. 88, 554-563.
5. Maundrell, K., Maxwell, E.S., Puvion, E. and Scherrer, K. (1981) Exp. Cell Res. 136, 435-445.
6. Nakayasu, H. and Ueda, K. (1981) Cell Struct. Funct. 6, 181-19o.
7. Hunt, B.F. and Vogelstein, B. (1981) Nuc. Acids Res. 9, 349-363.
8. Clawson, G.A. and Smuckler, E.A. (198o) Biochem. Biophys. Res. Comm. 96, 812-816.
9. Steer, R.C., Goueli, S.A., Wilson, M.J. and Ahmed, K. (198o) Biochem. Biophys. Res. Comm. 92, 919-925.
1o. Barrack, E.R. and Coffey, D.S. (198o) J. Biol. Chem. 255, 7265-7275.
11. Sevaljevic, L., Brajanovic, N. and Trajkovic, D. (1982) Molec. Biol. Rep. 8, 225-232.
12. Sevaljevic, L., Poznanovic, G., Petrovic, M. and Krtolica, K. (1981) Biochem. Int. 2, 77-84.
13. Allen, S.L., Berezney, R. and Coffey, D.S. (1977) Biochem. Biophys. Res. Comm. 75, 111-116.
14. Kostka, G. and Schweiger, A. (1981) Biochem. Biophys. Res. Comm. 1o1, 756-762.
15. Greenberg, J.R. and Setyono, B. (1981) Biol. Cell 41, 67-78.
16. Arnstein, H.R.V. (1982) Biochem. Soc. Symp. 47, 163-182.
17. Schweiger, A. and Kostka, G. (198o) Exp. Cell. Res. 125, 211-219.
18. Berezney, R. (198o) J. Cell Biol. 85, 641-65o.
19. Burton, K. (1956) Biochem. J. 62, 315-323.
2o. Ceriotti, D. (1955) J. Biol. Chem. 214, 59-7o.
21. Lowry, O., Rosebrough, N., Farr, A. and Randall, R. (1951) J. Biol. Chem. 193, 265-275.
22. Nakayasu, H., Mori, H. and Ueda, K. (1982) Cell Struct. Funct. 7, 253-262.