ACTA UNIVERSITATIS LODZIENSIS
FOLIA BIOCHIMICA ET BIOPHYSICA 14, 1999

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GLYCOPROTEINS ASSOCIATED WITH NUCLEAR MATRICES FROM HAMSTER, CHICKEN AND FROG LIVER CELLS: DETECTION AND CHARACTERIZATION

Nuclear matrices glycoproteins from hamster, chicken and frog liver cells were studied. Our results show the similarity in the profiles of liver nuclear matrices glycoproteins recognized by concanavalin A (ConA) of the examined animal species in contrary to the ones obtained after staining with *Galanthus nivalis* agglutinin (GNA). It may reflect the differences in the structure of oligosaccharide chains of some liver nuclear matrices glycoproteins from hamster, chicken and frog.

INTRODUCTION

Glycosylation is one of the major naturally occurring modifications of nuclear proteins covalent structure [17, 18, 20, 22, 31, 39]. A number of nuclear proteins such as nuclear pore complex proteins (designated nucleoporins) [31], RNA polymerase II [25] and some transcription factors [7, 23] were glycosylated in an unconventional way. They contained single *N*-acetylglucosamine (Glc*N*Ac) residues attached by *O*-linkage directly to serine and threonine residues at multiple sites. The specific functions of *O*-Glc*N*Acylation have not been elucidated. *O*-Glc*N*Acylation has been postulated to play a role in mediating assembly of multimeric protein complexes, in targeting proteins from the cytoplasm into the nucleus, in reversibly blocking sites of phosphorylation on serine or threonine residues of protein, in nucleus-cytoplasmic exchanges and in transcriptional regulation [16, 19, 20]. *O*-Glc*N*Acylation appears to be highly dynamic in a manner similar to protein phosphorylation [15, 20]. The Glc*N*Ac levels are regulated by the interplay of Glc*N*Ac transferase(s) and specific *N*-acetyl-β-D-glucosaminidase(s) [8, 13, 14]. The Glc*N*Ac transferase from rat liver cytosol capable of adding Glc*N*Ac to serine and threonine residues of protein and a neutral soluble *N*-acetyl-β-D-glucosaminidase from rat spleen

cytosol, which was highly efficient at removing Glc*N*Ac have been purified and characterized [8, 13, 14].

N-glycosylated proteins, in which oligosaccharides are attached to asparagine residues, have been identified as nuclear components not only in the nuclear envelope [3, 12, 40, 41], euchromatin fraction [24] but also in the nuclear matrix [4, 5, 10, 11, 29, 34]. The importance of *N*-bound oligosaccharide chains of nuclear matrix proteins for this structure was indicated by the results of the enzymatic deglycosylation experiments which showed solubilization of a significant fraction of matrix proteins (25%) on treatment of the matrix with *N*-glycosidase F [11]. Thus, it can be suggested that carbohydrate-protein interactions are additional factors, apart from disulfide cross-linkages, responsible for stabilization of the native nuclear matrix structure. The glycosylated HMG14 and 17 proteins belonging to high mobility group of nonhistone proteins bind preferentially to the nuclear protein matrix of mammalian cells [32]. Furthermore, this association appears to be mediated by the glycosyl chains since enzymatic removal of these modifications from the HMGs greatly reduced their binding to the nuclear matrix. It is tempting to suggest that this carbohydrate modification of HMG proteins may have functional significance for the architectural organization of the active domains of chromatin in cells.

The main aim of the present study was the detection and characterization of glycoproteins originating from liver nuclear matrices of the three animal species, i.e., hamster, chicken and frog.

MATERIALS AND METHODS

Tissues

Liver of three animal species: hamster (*Mesocricetus auratus*), chicken (*Gallus* sp.) and frog (*Rana esculenta*) were used for experiments.

Preparation of liver nuclei

All prepared solutions were used at 4°C. Liver nuclei from examined animal species were isolated by a modified sucrose method [6]. The tissue was homogenized in the proportions of 1g to 10 ml of 0.25 M sucrose, 5 mM $MgCl_2$, and 0.8 mM KH_2PO_4 at pH 6.7, filtered through four layers of gauze and spun down at 800x*g* for 7 min. The pellet was resuspended

in the above solution and Triton X-100 was added to a final concentration of 0.5% and then the suspension was homogenized again and centrifuged at 800xg for 7 min. The crude nuclear pellet was suspended in 10 vol 2.2 M sucrose, 5 mM $MgCl_2$ and centrifuged at 40,000x*g* for 45 min. The purity and integrity of the nuclei were checked by light microscopy.

Nuclear matrix isolation

Carefully purified nuclei from the above mentioned species (2-2.5 mg DNA/ml) were suspended in 0.25 M sucrose, 5 mM $MgCl_2$, 1 mM PMSF, 5 mM Tris-HCl buffer (pH 7.4) and digested by endogenous nucleases for 45 min at 37°C. The suspension was spun down at 7700x*g* for 15 min to separate nuclease-released products (NRP). The pellet was extracted three times with LS buffer: 0.2 mM $MgCl_2$, 1 mM PMSF, 10 mM Tris-HCl (pH 7.4) and three times with HS buffer: 2 M NaCl, 0.2 mM $MgCl_2$, 1 mM PMSF, 10 mM Tris-HCl (pH 7.4) at 4°C. The residual pellet was washed twice with LS buffer yielding nuclear matrices. Centrifugations were at 2000x*g* for 15 min during initial LS buffer extractions and at 7700x*g* for 15 min for all subsequent extractions [33, 37].

SDS-PAGE of proteins

For sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), nuclear matrices samples were mixed with 0.9 vol of solubilizing buffer (20% glycerol, 4% sodium dodecyl sulphate /SDS/, 25 μg pyronineY/ml, 0.125 M Tris-HCl buffer, pH 6.8) and 0.1 vol of 2-mercaptoethanol, and then heated in a boiling water bath for 5 min. One-dimensional electrophoresis was performed in slab polyacrylamide gels containing 0.1% SDS and 8% acrylamide (pH 8.8) with 3% stacking gel (pH 6.8) according to Laemmli [28] at 25 mA/slab gel until the pyronine Y marker reached the end of the stacking gel, and then at 35 mA/slab in the resolving gel until the marker dye reached the bottom of the gel. The gel slabs were stained with Coomassie brilliant blue R-250 according to Fairbanks et al. [9]. Excess of stain was removed with 10% acetic acid containing 5% methanol. Molecular masses of protein bands were calculated by comparison with the standard proteins: myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa) and carbonic anhydrase (29 kDa) (Sigma, St Louis, Missouri, U.S.A.).

Transfer of proteins from SDS gels

Proteins separated in SDS-polyacrylamide slab gels were transferred onto Immobilon-P transfer membrane (pore size 0.45 μm) by electrophoretic blotting in 20% methanol, 192 mM glycine, 25 mM Tris (pH 8.3) for 15 h at 60V and 4°C [36]. Proteins immobilized on transfer membranes were stained by a 5 min incubation at room temperature with 0.2% Ponceau S in 3% acetic acid, followed by destaining in H_2O until the protein bands were visible, in order to control the efficiency of blotting and to mark the location of standard proteins. The Ponceau S staining of the protein bands disappeared during subsequent incubation of the membrane in the blocking solution.

Detection of lectin-binding glycoproteins

The detection of lectin-binding glycoproteins immobilized on Immobilon-P sheets was accomplished by the method of Haselbeck et al. [21]. The membranes were treated for 30 min at room temperature with 20 ml 0.5% blocking reagent (w/v) in Tris buffered saline (TBS: 50 mM Tris-HCl, 150 mM NaCl, pH 7.5), and were washed twice with TBS and once with buffer one consisting of TBS, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 1 mM $CaCl_2$, at pH 7.5 (50 ml each). The membranes were incubated for 1 h at room temperature with the lectin-digoxigenin (DIG) conjugate (GNA, *Galanthus nivalis* agglutinin, 1 μg/ml; ConA, Concanavalin A, 10 μg/ml) in 20 ml of buffer one.

Then, the membranes were washed three times with 50 ml TBS, and polyclonal sheep anti-digoxigenin Fab fragments conjugated with alkaline phosphatase (anti-DIG AP, 750 U/ml) were added in a 1:1,000 dilution in 20 ml TBS and incubated for 1 h at room temperature. The membranes were again washed three times with 50 ml TBS and the alkaline phosphatase reaction was carried out by incubating the membrane without shaking in 20 ml of the following freshly prepared solution: 75 μl 5-bromo-4-chloro-3--indolyl phosphate 4-toluidine salt (BCIP 50 mg/ml, in dimethylformamide) and 100 μl 4-nitro blue tetrazolium chloride (NBT 75 mg/ml, in 70% dimethylformamide) in 20 ml of buffer two (100 mM Tris-HCl, 50 mM $MgCl_2$, 100 mM NaCl at pH 9.5). The reaction was completed within a few minutes. The membranes were raised with H_2O to stop the reaction and allowed to dry.

Analytical procedures

Protein was estimated using bovine serum albumin as a standard by the method of Lowry et al. [30]. DNA concentration was determined from the absorbance at 260 nm (1 mg DNA/ml corresponds to 20 O.D. units) of samples dissolved in 5 M ultra-pure urea.

Reagents

Digoxigenin-labelled lectins, blocking reagent and polyclonal sheep anti-digoxigenin Fab fragments conjugated with alkaline phosphatase, used for the detection and characterization of glycoproteins, were obtained from Boehringer Mannheim, Germany. For blotting the Immobilon-P transfer membrane (pore size 0.45 μm) obtained from Millipore Corporation, Bedford, Massachusetts, U.S.A., was used. Other chemicals were of the highest available purity and were from Sigma, St Louis, Missouri, U.S.A.

RESULTS AND DISCUSSION

The nuclear matrix is operationally defined as the residual nuclear structure that is yielded by sequential treatment of isolated nuclei with detergents, nucleases and buffers of high ionic strength (for reviews see [1, 2, 26, 27]). The isolated nuclear matrix is composed of three major electron microscopically identifiable structural domains, namely: the surrounding pore complex-lamina, residual nucleoli and an internal fibrogranular matrix. The nuclear matrix represents the three-dimensional fibrillar protein structure constituting the framework of the interphase nucleus. In addition to its role in maintaining the nuclear architecture and the higher order structure of chromatin, the nuclear matrix has been reported as being involved in various nuclear activities such as DNA replication, DNA transcription, RNA processing and steroid-hormone action [1, 26, 38]. It was stated that some nuclear matrix proteins are cell-, tissue-, differentiation- and tumor-specific [2, 35]. Moreover, evidence was demonstrated for the presence of a common set of polypeptides in the nuclear matrices of various cell types [1, 2]. Although the presence of glycoproteins associated with the nuclear matrix was described by many authors [4, 5, 10, 11, 29, 34], until now their characterization from nuclear matrices of different animal species has been very limited.

The present study concerns the *N*-glycosylated proteins recognized by ConA and GNA from liver nuclear matrices of three animal species, i.e., hamster, chicken and frog. Nuclei from liver cells of these vertebrates were carefully isolated and checked for integrity and purity by observation with light microscopy. Liver nuclear matrices from the purified nuclei of the examined species were obtained by the technique developed in Berezney's laboratory [33, 37]. Nuclear matrix proteins were separated by one-dimensional electrophoresis performed in slab polyacrylamide gels containing 0.1% SDS and 8% acrylamide (pH 8.8) with 3% stacking gel (pH 6.8) according to Laemmli [28]. Typical polypeptide profiles of liver nuclear matrices of the examined animal species obtained by staining with Coomassie brilliant blue R-250 are shown in Fig. 1. The proteins of whole nuclear matrices separated by SDS-polyacrylamide slab gel electrophoresis (Fig. 1) were transferred onto Immobilon-P membrane and tested for DIG-ConA and DIG-GNA binding (see Materials and Methods). The effects of these experiments are demonstrated in Fig. 2. The resulting patterns shown in Fig. 2A allow to compare the nuclear matrices glycoproteins recognized by ConA from liver of hamster, chicken and frog. The detailed analysis of all separations indicated the similarity in the profiles of the main glycoproteins. However, the limitation which was given by used system of electrophoresis makes impossible the comparison of glycoproteins existing as minor components.

It is remarkable, that the patterns of liver nuclear matrices glycoproteins recognized by ConA of examined species differed significantly from those obtained after staining with GNA – a lectin specific for terminal bound mannoses (α1-2, 1-3, 1-6 to mannose) (Fig. 2B). At least six glycoproteins of nuclear matrix of hamster liver stained by ConA were recognized also by GNA. It was observed that GNA reacts strongly with glycoproteins with molecular mass to about 180 and 46 kDa. In the case on nuclear matrix glycoproteins of chicken liver seven of them occurring between 46 and 158 kDa were stained with GNA as well as with ConA. From six glycoproteins of nuclear matrix from frog liver the major GNA binding protein has an approximate molecular mass of 60/62 kDa.

The results presented in this study indicate that the differences between glycoproteins associated with nuclear matrices of the three animal species may be attributed to a different level of their glycosylation. It is possible that the differences in the structure of oligosaccharide chains of nuclear matrices glycoproteins from different animal species may affect diversity of their function.

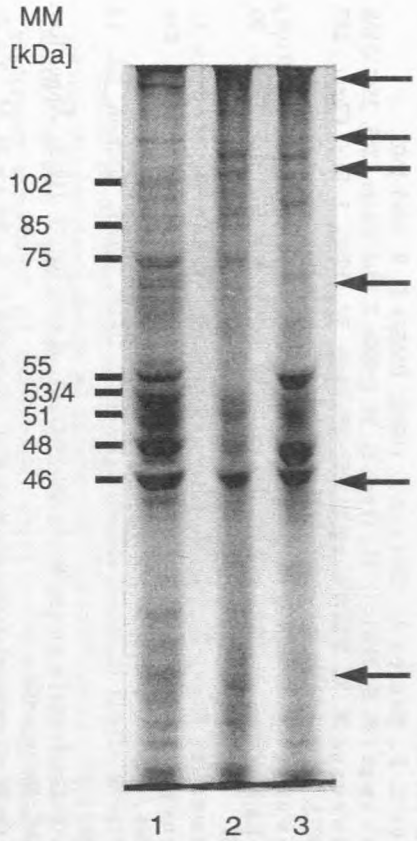

Fig. 1. SDS-PAGE of liver nuclear matrices proteins from hamste (1), chicken (2) and frog (3) on 8% acrylamide slab gel. Arrows indicate the positions of marker proteins of 205, 116, 97, 66, 45 and 29 kDa from top to bottom. Gel was stained with Coomassie brilliant blue R-250. About 50 μg proteins were applied per gel

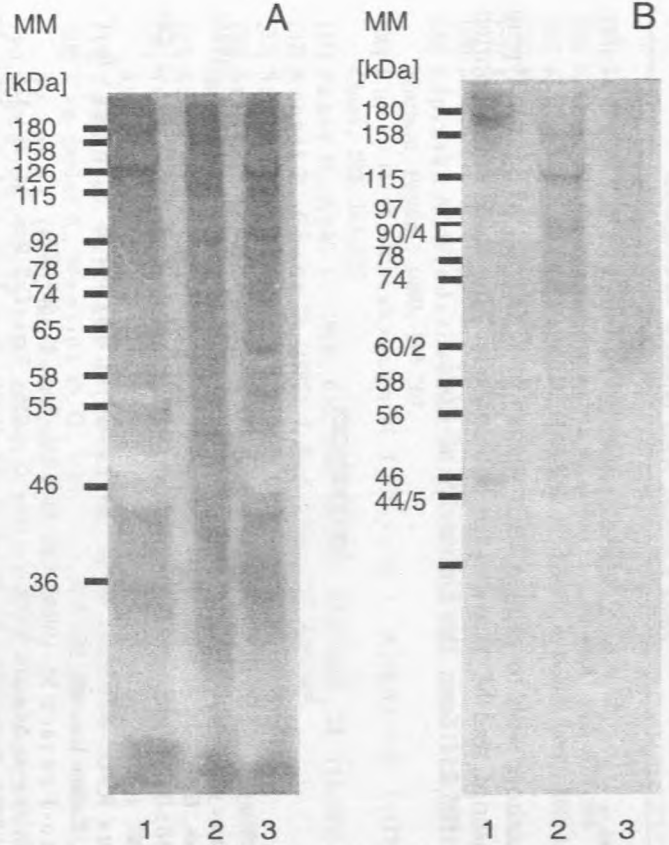

Fig. 2. Liver nuclear matrices proteins from hamster (1), chicken (2) and frog (3) electrophoresed on 8% acrylamide slab gel were transferred to Immobilon-P membranes and tested for DIG-ConA (A) and DIG-GNA (B) binding

ACKNOWLEDGEMENTS

The authors wish to express their thanks to J. Gierak, M.Sc. for typing the manuscript, and M. Radwan for photography. This work was supported by grant 505/254 from the University of Łódź.

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Wpłynęło do Redakcji
Folia biochimica et biophysica
24.04.1998

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GLIKOPROTEINY ZASOCJOWANE Z MATRIKS JĄDROWĄ KOMÓREK WĄTROBY CHOMIKA, KURY I ŻABY: IDENTYFIKACJA I CHARAKTERYSTYKA

Badano glikoproteiny matriks jądrowej wątroby chomika, kury i żaby. Nasze wyniki wykazują podobieństwo wykresów glikoprotein rozpoznawanych przez konkanawalinę A (ConA) matriks jądrowej wątroby badanych gatunków zwierząt w przeciwieństwie do wykresów glikoprotein otrzymanych po zastosowaniu lektyny z *Galanthus nivalis* (GNA). Może to odzwierciedlać różnice w strukturze łańcucha oligosacharydowego pewnych glikoprotein matriks jądrowej wątroby chomika, kury i żaby.