Identification of Two Nuclear N-Acetylglucosamine-Binding Proteins

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Abstract   Using neoglycoproteins, lectins that recognize different sugars, including N-acetylglucosamine residues, were previously detected in animal cell nuclei. We report herein the isolation of two N-acetylglucosamine-binding proteins from HL60 cell nuclei: i) a 22 kDa polypeptide (CBP22) with an isoelectric point of 4.5 was isolated for the first time and ii) a 70 kDa polypeptide with an isoelectric point of 7.8. This latter protein corresponds to the glucose-binding protein (CBP70) previously isolated, based on the following similarities: i) they have the same molecular mass, ii) they have the same isoelectric point, iii) they are recognized by antibodies raised against CBP70, and iv) both are lectins from the C group of Drickamer's classification. CBP70 appeared to recognize glucose and N-acetylglucosamine; however, its affinity for N-acetylglucosamine was found to be twice that for glucose. The presence in the nucleus of two nuclear N-acetylglucosamine-binding proteins and their potential ligands, such as O-N-acetylglucosamine glycoproteins, strongly argues for possible intranuclear glycoprotein-lectin interactions.   © 1994 Wiley-Liss, Inc.

Key words: lectin, nucleus, HL60, affinity chromatography, glycoprotein

Since the presence of lectins in the cell nucleus was demonstrated [Hubert et al., 1985; Sève et al., 1985, 1986], four nuclear lectins have been characterized: two galactose/lactose-binding proteins, CBP35 [Roff and Wang, 1983], and CBP14 [Cuperlovic et al., in press], isolated from 3T3 fibroblasts and rat liver nuclei, respectively, and two glucose-binding proteins, CBP67 [Schröder et al., 1992] and CBP70 [Sève et al., 1993], isolated from rat liver nuclei and HL60 cell nuclei, respectively. Most of these polypeptides are associated with ribonucleoprotein complexes [Sève et al., 1986; Laing and Wang, 1988; Schröder et al., 1992]. Although their roles remain to be specified, some preliminary data suggest that CBP67 and CBP14 might be involved in the nucleocytoplasmic transport of mRNA [Schröder et al., 1992; Cuperlovic et al., 1993], while CBP35 could participate in RNA splicing [Wang et al., 1991b]. It has been thought [Hubert et al., 1989] that nuclear lectins might play their role

by interacting with the sugar moieties of glycoproteins which are also known to be present in the nucleus. Although there is a line of evidence suggesting the existence of nuclear glycoproteins containing N-linked complex-oligosaccharide chains [Stein et al., 1975, 1981; Ferraro et al., 1991; Codogno et al., 1992], the only well-characterized nuclear glycoproteins bear N-acetylglucosamine residues attached in O-linkages (termed O-GlcNAc) [Holt and Hart, 1986; reviewed in Haltiwanger et al., 1991]. Some results obtained in vitro by different groups support the possible involvement of interactions between these glycoproteins and endogenous lectins in nuclear activities. For instance, the vegetal lectin, Wheat Germ Agglutinin (WGA), inhibits active nucleocytoplasmic exchanges [Finlay et al., 1987] and the transcriptional activation function of Sp1 [Jackson and Tjian, 1988] when it binds to GlcNAc residues borne by nuclear-pore proteins and Sp1. Furthermore, recent findings suggested that some glycoprotein-lectin interactions could modify protein-protein interactions [Sève et al., in press]. Nevertheless, the concept that glycoprotein-lectin interactions could occur in the cell nucleus remained still purely speculative, since the only

nuclear lectins isolated so far recognize lactose (Lac) or glucose (Glc) residues, while only O-GlcNAc nuclear proteins have been clearly identified. As emphasized in a recent review [Wang et al., 1991a], even if the presence of lectins able to recognize GlcNAc residues could be suspected from the binding of GlcNAc-bearing neoglycoproteins to isolated nuclei [Sève et al., 1986], identification of these nuclear lectins remained a future challenge.

In the present report, we describe the identification, for the first time, of two GlcNAc-binding proteins, referred to as CBP22 and CBP70, in the nuclei of human myeloid leukemia cells.

MATERIALS AND METHODS
Cell Culture

HL60 cells (provided by Dr. Degos, Hôpital Saint-Louis, Paris, France) were grown in suspension in RPMI 1640 medium (Gibco) supplemented with 10% heat-inactivated fetal calf serum (Gibco), 2 mM L-glutamine, 50 IU/ml of penicillin, and 50 μg/ml of streptomycin. Cells were incubated at 37°C in a humidified 5% CO_2 atmosphere and maintained at a density of 2 × 10^5 to 1 × 10^6 cells/ml by resuspending them in fresh culture medium every 3 days. Cells were used between passages 15 and 40.

Isolation of Membrane-Depleted Nuclei

Very clean membrane-depleted nuclei were isolated from HL60 cells according to a previously reported method [Facy et al., 1990]. Briefly, exponentially growing HL60 cells (1 × 10^9 cells) were washed twice in 10 mM Tris-HCl (pH 7.5) containing 20 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 0.2 mM spermidine (TKCM buffer), and collected by low-speed centrifugation (800g for 10 min). The cell pellets were resuspended in 20 ml of TKCM buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml each of antipain, leupeptin, pepstatin A, and chymostatin, 0.17 IU/ml of aprotinin, and 20 mM benzamidine as protease inhibitors (Sigma Chemical Co., St. Louis, MO). Triton X-100 was added to a final concentration of 0.5%. Cells were homogenized and membrane-depleted nuclei were pelleted by centrifugation at 1,000g for 10 min. The pellets were washed twice in 20 ml of TKCM buffer and pelleted again by centrifugation. All steps of the isolation procedure were carried out at 4°C.

Nuclear Protein Extraction

Membrane-depleted nuclei were resuspended in TKCM buffer adjusted to 2 M NaCl (final concentration). Protein extraction was carried out for 1 h at 4°C. The excess NaCl was removed by dialysis overnight against Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS) at 4°C, as previously described [Sève et al., 1993]. The supernatant containing extracted proteins was concentrated on Centricon-10 filters (Grace and Co., Danvers, MA).

Radiolabeling of Nuclear Proteins

Extracted proteins were radiolabeled with [125]I (sp. act. 3.7 GBq/ml) using the iodogen method [Fraker and Speck, 1978]. The labeled proteins were dialyzed against PBS containing 0.7 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (complete PBS) at 4°C for 6 h to eliminate free iodine.

Affinity-Chromatography Procedures

N-acetyl-β-D-glucosamine phenylisothiocyanate (GlcNAc-φ-NCS) and α-D-glucopyranosyl-isothiocyanate (Glc-φ-NCS) (Sigma Chemical Co., St. Louis, MO) were immobilized on acetone-dehydrated Trisacryl GF 2000 M (IBF). The nuclear protein solution was adjusted to 0.7 mM $CaCl_2$ and 0.5 mM $MgCl_2$ and affinity chromatography was performed as previously described [Sève et al., 1993] but, in some experiments, the proteins were incubated with the chromatography matrix in the absence of divalent cations. The elution was performed with 0.2 M heterologous sugars, such as fucose and galactose, before the addition of 0.2 M homologous sugar (Glc or GlcNAc). To test the sugar specificity of the lectins, eluted and unbound protein fractions were collected from Glc and GlcNAc columns and subsequently incubated with GlcNAc and Glc columns, respectively.

When radiolabeled nuclear proteins were used, 1 ml fractions were collected at a flow rate of 300 μl/min and radioactivity was measured in a γ counter (Compugamma 1282, LKB, Bromma, Sweden).

Detection of N-Acetylglucosamine-Binding Sites in Nuclear Protein Extracts

The presence of GlcNAc-binding components in protein fractions was tested after electrophoresis. For this purpose, unlabeled fractions containing GlcNAc-binding proteins, based on their specific elution from affinity-chromatography

columns, were used as follows: to remove free sugars, the proteins were dialyzed against complete PBS, and then concentrated and mixed in the migration buffer according to the Laemmli's procedure [1970], except that β-mercaptoethanol was omitted and the proteins were not boiled but heated at 37°C for 20 min. Then, proteins were loaded onto the gel in three different lanes. After migration, the proteins were renaturated, according to Blanck et al. [1982], by removing SDS by two successive 15 min washes in 10 mM Tris-HCl (pH 7.5) containing 5 mM β-mercaptoethanol and 20% isopropanol at room temperature. After this treatment, the gel was soaked in 50 mM Tris-HCl (pH 7.5) containing 5 mM β-mercaptoethanol overnight at 4°C. It was subsequently incubated in 25 mM Tris-HCl (pH 7.8) containing 0.5 M NaCl and 1% non-fat milk powder (Régilait, France), for 2 h at room temperature and finally washed with complete PBS containing 1% milk powder. Each of the three lanes was cut into 24 separate pieces and each one was placed sequentially in a test tube. N-acetyl-β-D-glucosaminylated bovine serum albumin (GlcNAc-BSA) and BSA were radioiodinated according to the chloramine-T procedure [Hunter and Greenwood, 1962]. Briefly, 100 mg of chloramine-T (MERCK) and 2×10^7 Bq of ^{125}I Na were added to 1 mg of BSA or GlcNAc-BSA. The reaction was stopped by adding sodium metabisulfite at a final concentration of 2 mg/ml and free iodine was eliminated by gel filtration on a 10 ml GF05 column (IBF) equilibrated with complete PBS. The first-lane pieces were incubated with ^{125}I-GlcNAc-BSA (10 mg/ml) for 2 h. To control the specificity, the second-lane slices were incubated with ^{125}I-BSA (10 mg/ml) for 2 h and the third-lane segments were preincubated with unlabeled GlcNAc-BSA (2 mg/ml) for 2 h and then incubated with ^{125}I-GlcNAc-BSA (10 mg/ml) for 2 h. All these steps were performed at room temperature. After extensive washing in complete PBS, the samples were analyzed for radioactivity contents. The relationship between the radioactivity profile and the electrophoretic pattern of proteins was established by reference to a lane of gel containing molecular mass markers run in parallel with the nuclear proteins and stained with Coomassie brilliant blue R250.

Electrophoresis Analysis

The polypeptides were resolved by Sodiumdodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 12% acrylamide mini-slab running gels under denaturing conditions, according to Laemmli's procedure [1970]. The polypeptide bands were visualized by silver staining.

Immunoblotting Analysis

Polypeptides, resolved by SDS-PAGE on 12% acrylamide mini-slab running gels, were transferred electrophoretically onto Immobilon-P paper (Millipore, Bedford, MA) (60 V at room temperature for 1 h) in 10 mM (cyclohexylamino)-1-propane sulfonic acid, pH 10.7 (Caps buffer). The Immobilon paper was incubated overnight in saturating TBS buffer (20 mM Tris-HCl (pH 7.4), 0.5 M NaCl) containing 5% milk powder. The paper was then washed three times in TBS containing 0.5% Tween 20 (TBS-Tween) and incubated for 3 h with rabbit antiserum raised against CBP70 (anti-CBP70), obtained as previously described [Sève et al., 1993] and diluted 1:1,000 in TBS-Tween or with rabbit antiserum raised against CBP35 (anti-CBP35-70) (generously provided by Pr. J.L. Wang), diluted 1:750 in TBS-Tween. This antiserum was found to cross-react with CBP35 and CBP70 in HL60 cell nuclear extracts [Sève et al., 1993] and is therefore referred to as anti-CBP35-70 antiserum in this text. After incubation in the presence of antibodies, the paper was washed three times in TBS-Tween, incubated for 1 h at 4°C with alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma, France) at a dilution of 1:8,000, and then washed three times in TBS-Tween and finally in Tris buffer (0.1 M Tris-HCl (pH 9.5), 100 mM NaCl, and 5 mM MgCl$_2$) before staining with 5-bromo 4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (NBT) (Sigma Chemical Co., St. Louis, MO) as substrates. Controls were run to assure the lack of non-specific binding of alkaline phosphatase-conjugated goat anti-rabbit IgG and the absence of endogenous alkaline phosphatase.

The staining intensity of immunoblot bands, reported in arbitrary units, was quantified by immunoblot densitometric scanning.

Protein Elution From SDS-PAGE

Proteins, eluted from GlcNAc columns, were resolved by SDS-PAGE on 12% acrylamide gels under denaturing conditions according to Laemmli's procedure [1970]. The polypeptide bands were visualized with 0.5% Coomassie brillant blue R250 in acetic acid, isopropylic alcohol

and H_2O (1:3:6). The incubation was performed for 15 min at room temperature. After staining, the gel was incubated with destaining solution until only the polypeptide bands remained colored. The gel was then washed four times in water and the protein bands were excised. These bands were homogenized in Eppendorf tubes and suspended in 0.05 M ammonium carbonate buffer containing 0.1% SDS and 5% β-mercapto-ethanol overnight at 37°C. After elution, the treated gel was centrifuged (2,000g for 10 min) and the supernatant containing proteins was dialyzed against water for 72 h.

Isoelectric Focusing (IEF)

CBP70, eluted from a Glc column and solubilized from SDS-PAGE as described above, and a GlcNAc-eluted fraction (4 μl samples diluted 1:1 in 8 M urea) were focused on PhastGels IEF 3-9 (Pharmacia LKB, Sweden) as previously described [Avellana-Adalid et al., 1992]. pI calibration kits (pH range covered 3-9; Pharmacia IEF 3-9) were used as standards [amyloglucosidase (pI 3.5), soybean trypsin inhibitor (pI 4.55), β-lactoglobulin A (pI 5.2), bovine carbonic anhydrase B (pI 5.85), human carbonic anhydrase B (pI 6.55), myoglobin acidic band (pI 6.85), myoglobin basic band (pI 7.35), lentil-lectin acidic band (pI 8.15), lentil-lectin middle band (pI 8.45), lentil-lectin basic band (pI 8.65), and trypsinogen

(pI 9.3)] and proteins were silver-stained according to the manufacturer's instructions.

RESULTS

Elution Profile of a Nuclear Extract Subjected to N-Acetylglucosamine-Affinity Chromatography

To define the optimal conditions for GlcNAc-affinity chromatography, the elution profile was analyzed using a radiolabeled nuclear extract. After removing the unbound material by washing the columns with complete PBS, the radioactivity was very low (Fig. 1a). The addition of 0.2 M L-fucose or 0.2 M D-galactose failed to release any radioactive material (Fig. 1b,c), whereas the radioactivity sharply increased in the fractions collected immediately after the addition of 0.2 M GlcNAc (Fig. 1, peak d), and then quickly dropped to the background level. Subsequent elution with 0.4 M GlcNAc did not release any more radioactivity (Fig. 1e), indicating that 0.2 M GlcNAc was sufficiently concentrated to cause the release of the polypeptides bound to the column.

Detection of N-Acetylglucosamine-Binding Sites After Electrophoresis of the Nuclear Extract

Gel slices containing non-labeled proteins eluted with 0.2 M GlcNAc were incubated in the presence of radiolabeled GlcNAc-BSA and the radioactivity profile, obtained by counting each gel piece individually, showed a peak correspond-
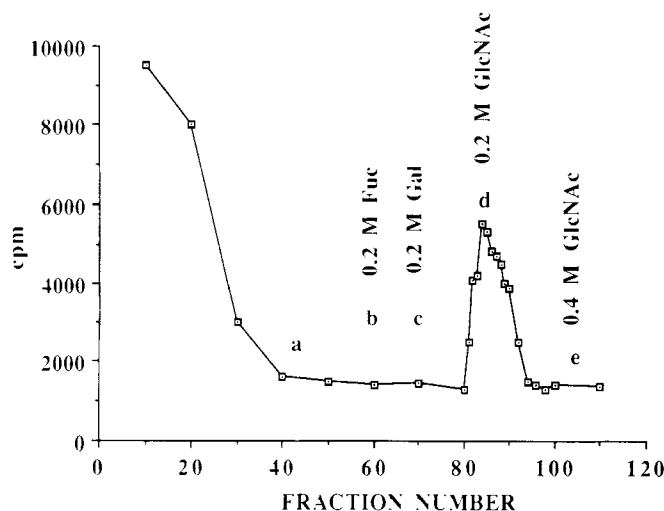

Fig. 1. Elution of an ^{125}I-labeled nuclear extract of HL60 cells subjected to GlcNAc-affinity chromatography; 5 mg/ml of protein were loaded onto the column. After removing the unbound material (a), 0.2 M of L-fucose (Fuc) or D-galactose (Gal) were added to the GlcNAc-affinity column (b and c), before the addition of 0.2 M GlcNAc (peak d) and then 0.4 M GlcNAc (e).
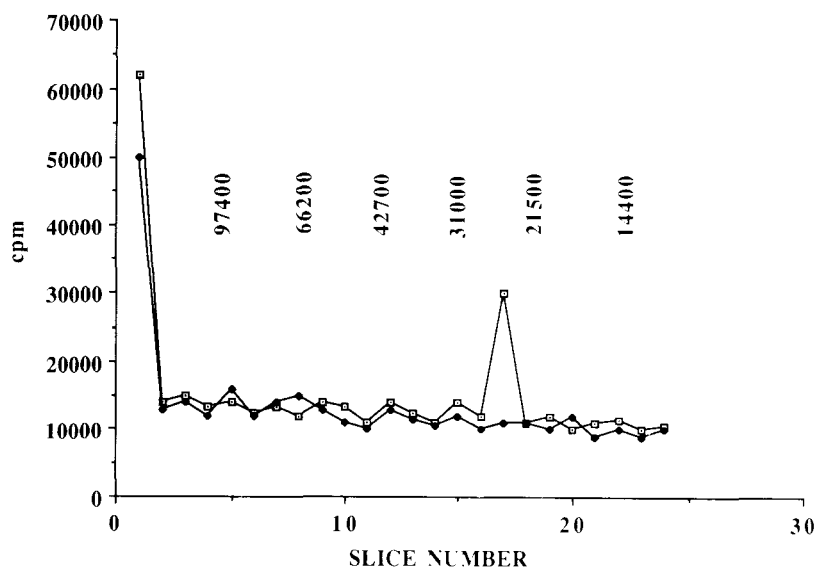
Fig. 2. Detection of GlcNAc-binding sites after SDS-PAGE. Non-radioactive eluted proteins were not treated with β-mercaptoethanol or boiled before electrophoresis. After protein migration, each of the three lanes of the gel was cut into 24 consecutive slices of 0.25 cm². The first-lane pieces were incubated with ^{125}I-GlcNAc-BSA, the second-lane segments with ^{125}I-BSA, and the third-lane slices were preincubated with GlcNAC-BSA and then incubated with ^{125}I-GlcNAc-BSA. (————□————) ^{125}I-labeled GlcNAc-BSA. (————■————) Controls: ^{125}I-labeled BSA or GlcNAc-BSA.

ing to slice number containing 22 kDa polypeptide, whereas no radioactivity was detected in the other pieces of the gel (Fig. 2).

When the gel pieces were preincubated with unlabeled GlcNAc-BSA, no radioactivity peak was observed. The same result was obtained when serial gel slices were incubated with radioiodinated BSA alone (Fig. 2), thereby demonstrating the specificity of the labeling with ^{125}I-GlcNAc-BSA.

Identification of the N-acetylglucosamine-Binding Polypeptides

The 0.2 M NaCl-extracted nuclear material that bound to GlcNAc columns and was eluted by 0.2 M GlcNAc (GlcNAc-eluted fraction) contained two predominant polypeptides of 70 kDa and 22 kDa, visualized after SDS-PAGE and silver staining (Fig. 3A, lane 3). The two polypeptides were missing when all the steps of the affinity chromatography were performed without any divalent cations (Fig. 3A, lane 2). The application of 0.2 M heterologous sugars, such as fucose or galactose, before 0.2 M GlcNAc did not release any polypeptides (Fig. 3B, lanes 1 and 2).

The presence of the two polypeptides in the GlcNAc-eluted fraction could be due to either the interaction between the polypeptides or the presence of a GlcNAc-binding site in both poly-

peptides. To clarify this point, the polypeptides were solubilized from the gels and then each one was incubated with a GlcNAc column and resubjected to SDS-PAGE. The 70 kDa polypeptide was not found in the GlcNAc-eluted fraction (Fig. 4, lane 2), while it was identified in the unbound protein fraction (Fig. 4, lane 3). In contrast, the totality of the 22 kDa polypeptide was retained on the column (Fig. 4, lane 4), since no polypeptide was found in the unbound protein fraction (Fig. 4, lane 5). These results are therefore in agreement with those obtained when GlcNAc-binding sites were studied with ^{125}I-GlcNAc-BSA. In both cases, unlike the 70 kDa, the 22 kDa polypeptide was unambiguously identified as a GlcNAc-binding protein. Concerning the 70 kDa polypeptide, it could logically be thought that an interaction between this polypeptide and the 22 kDa polypeptide could occur because the 70 kDa polypeptide was neither retained on the GlcNAc column after elution from the gel nor recognized after the incubation of the gel with ^{125}I-GlcNAc-BSA. Additionaly, the 70 kDa polypeptide, which has the same molecular mass as the CBP70 isolated under Glc-affinity chromatography conditions [Sève et al., 1993], could correspond to this Glc-binding protein.

To test these hypotheses, GlcNAc- and Glc-eluted fractions were immunoblotted with anti-CBP70 antiserum. Positive reactions on the 70
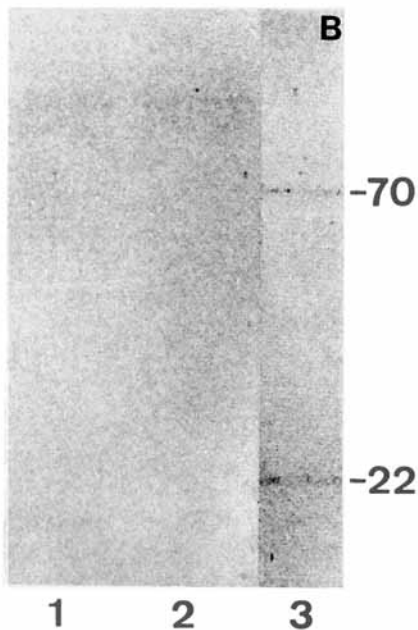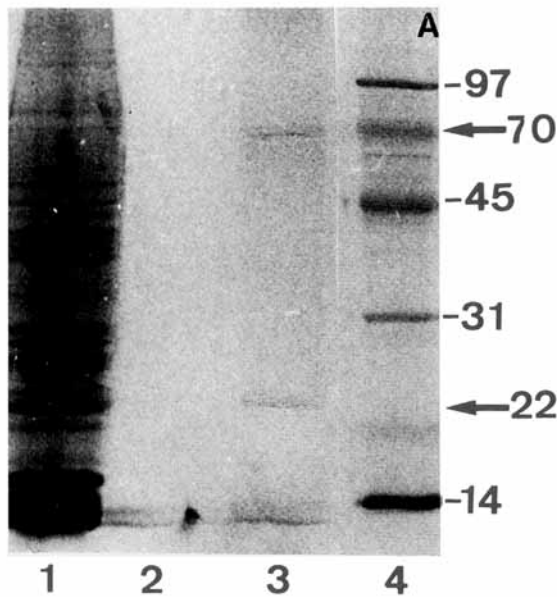
Fig. 4. GlcNAc-affinity chromatography of the 22 kDa and 70 kDa polypeptides solubilized from SDS-12% PAGE. Polypeptides were resolved on SDS-12% PAGE and silver stained. Molecular mass standards (lane 1). GlcNAc column incubated with the 70 kDa polypeptide and eluted with 0.2 M GlcNAc (lane 2). Unbound protein fraction (lane 3). GlcNAc column incubated with the 22 kDa polypeptide and eluted with 0.2 M GlcNAc (lane 4). Unbound protein fraction (lane 5).

Fig. 3. Electrophoretic analysis of polypeptides bound to a GlcNAc column; 5 mg of protein were subjected to affinity chromatography and the eluted proteins were resolved on SDS-12% PAGE and silver stained. **A:** 2 M NaCl extract of membrane-depleted nuclei (lane 1). Specific elution with 0.2 M GlcNAc in the absence of divalents cations (lane 2). Specific elution with 0.2 M GlcNAc in the presence of divalents cations (lane 3) Molecular mass standards (lane 4). **B:** Heterologous elution with 0.2 M Fuc (lane 1). Heterologous elution with 0.2 M Gal (lane 2). Homologous elution with 0.2 M GlcNAc (lane 3).

kDa band were observed in the Glc-eluted fraction (Fig. 5, lane 1) and in the GlcNAc-eluted fraction (Fig. 5, lane 2). However, because a cross-reaction between the antibodies and another polypeptide could not be excluded, the
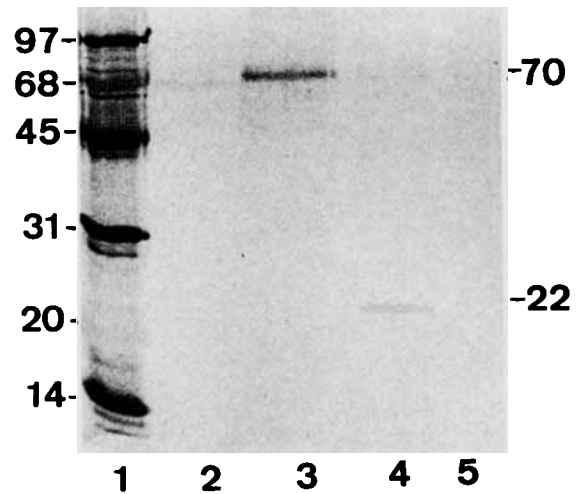
fraction eluted from a Glc column was incubated on a GlcNAc column. In this case, the immunoblotting analysis with anti-CBP70 antiserum revealed that the 70 kDa polypeptide was present in the GlcNAc-eluted fraction (Fig. 6, lane 1) and absent in the unbound protein fraction (Fig. 6, lane 2). On the other hand, when the eluted fraction from a GlcNAc column was incubated on a Glc column, the immunoblotting analysis revealed the 70 kDa polypeptide in the Glc-eluted fraction (Fig. 6, lane 3) and not in the unbound protein fraction (Fig. 6, lane 4). Densitometric scanning of the immunoblot showed that the labeling intensity with anti-CBP70 antiserum was almost two times stronger (40%) in the GlcNAc-eluted fraction than in the Glc-eluted fraction. Moreover, the 70 kDa polypeptide of the GlcNac-eluted fraction was recognized by anti-CBP35-70 antiserum, which recognizes CBP70 eluted from a Glc column [Sève et al., 1993], but no CBP35 was detected (Fig. 7, lane 1). Taken together, these observations strongly suggested that the 70 kDa polypeptide corresponds to CBP70. In addition, this relationship was supported by the results of the isoelectric focusing of the GlcNAc-eluted fraction, which revealed two polypeptides: one with the same pl (7.8) as CBP70 and another of pl 4.5, which could be attributed to the 22 kDa polypeptide (data not shown). Thus, the Glc-binding
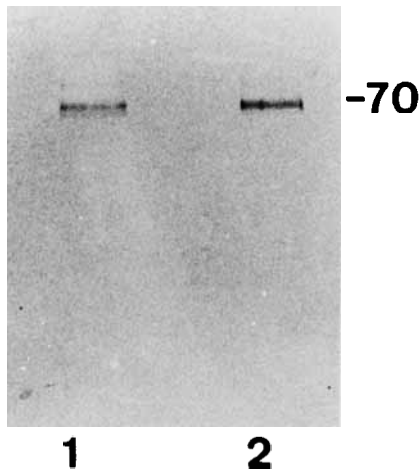
Fig. 5. Immunoblotting analysis of the polypeptides eluted from Glc and GlcNAc columns with polyclonal antibodies. Polypeptides were eluted from the Glc column with 0.2 M Glc (lane 1) and from the GlcNAc column with 0.2 M GlcNAc (lane 2) and labeled with anti-CBP70 antiserum.
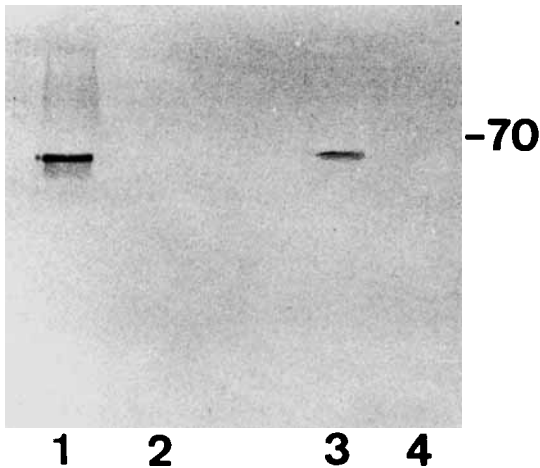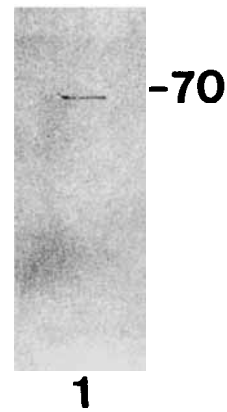

Fig. 7. Immunoblotting analysis of the polypeptides eluted from a GlcNAc column with anti-CBP35-70 antiserum. Proteins were eluted from a GlcNAc column with 0.2 M GlcNAc (lane 1).

Fig. 6. Bands were labeled with anti-CBP70 antiserum. GlcNAc-affinity chromatography of the polypeptides eluted from a Glc column (lane 1). Unbound protein fraction of the GlcNAc column (lane 2). Glc-affinity chromatography of the polypeptides eluted from a GlcNAc column (lane 3). Unbound protein fraction of the Glc column (lane 4).
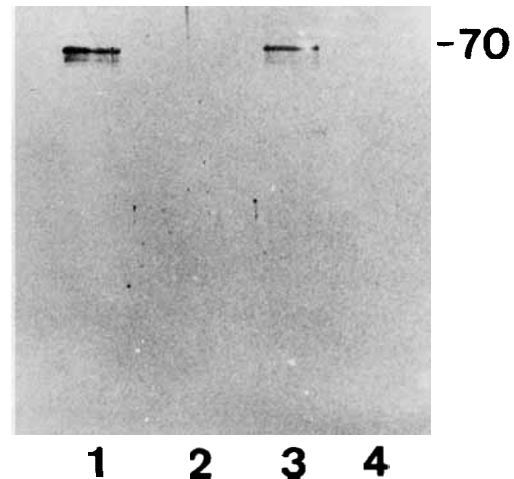

Fig. 8. Anti-CBP70 antiserum-labeling of heterologous fractions eluted from a Glc column, first by 0.2 M GlcNAc (lane 1), and then by 0.2 M Glc (lane 2) and those eluted from a GlcNAc column, first by 0.2 M Glc (lane 3), and then by 0.2 M GlcNAc (lane 4).

protein CBP70 appeared also to be a GlcNac-binding protein rather than a protein retained on GlcNAc column in association with CBP20. The capacity of this lectin to recognize both Glc and GlcNAc was confirmed by the results of heterologous elutions from Glc and GlcNAc columns with 0.2 M GlcNAc and Glc, respectively, before homologous elutions. Indeed, when Glc column was first treated with GlcNAc, CBP70 was eluted as shown by immunoblotting analy-

sis with anti-CBP70 antiserum (Fig. 8, lane 1). A similar result was obtained when GlcNAc column was preeluted with 0.2 M Glc (Fig. 8, lane 3). In both cases, the subsequent addition of the homologous sugar did not release any polypeptides (Fig. 8, lanes 2 and 4).

In contrast, when the fraction specifically eluted from a GlcNAc column was then incubated on a Glc column, the 22 kDa polypeptide was visualized only in the unbound-protein fraction (data not shown). Furthermore, this polypeptide was detected in the GlcNAC-eluted fraction after incubation of the unbound-protein fraction from a Glc column on a GlcNAc column.

DISCUSSION

The results reported here demonstrate the presence of two GlcNAc-binding proteins in animal cell nuclei. One is a 22 kDa polypeptide and the other is a 70 kDa polypeptide and both belong to the C group of lectins according to Drickamer's classification [Drickamer, 1988]. The 22 kDa polypeptide (CBP22) has been isolated for the first time. In contrast, the 70 kDa polypeptide appears to be the CBP70 previously isolated from HL60 cell nuclei and identified as a Glc-binding protein [Sève et al., 1993]. Indeed, the two polypeptides i) have the same molecular mass, ii) do not bind to GlcNAc or Glc columns in the absence of divalent cations, iii) have the same isoelectric point 7.8, and iv) are recognized by anti-CBP70 and anti-CBP35-70 antibodies. Furthermore, the 70 kDa polypeptide eluted from a GlcNAc column bound subsequently to a Glc column. Similarly, the 70 kDa polypeptide present in the fraction eluted from a Glc column was retained on a GlcNAc column. Finally, the 70 kDa polypeptide could be eluted, from GlcNAc or Glc columns, by the addition of Glc or GlcNAc, respectively. It should be noted that CBP70 lost its GlcNAc-binding ability when it was subjected to electrophoresis, even if this procedure was performed under semi-denaturing conditions. This result strongly suggests that the carbohydrate-recognition domain (CRD) of CBP70 undergown non-reversible denaturation during electrophoresis.

Surprisingly, no CBP35 was detected in the GlcNAc-eluted fraction, despite the identification of its association with CBP70 by a protein-protein interaction during Glc-affinity chromatography [Sève et al., 1993]. Moreover, CBP35-CBP70 interaction was found to be altered, probably as a consequence of a tridimensional modification of CBP35 upon lactose binding to its CRD [Sève et al., 1993], while galactose, which is recognized with a lower affinity, did not have the same effect. It can therefore be hypothesized that CBP35-CBP70 interactions might be disrupted when CBP70 binds to GlcNAc. However, further investigations are necessary to confirm this hypothesis.

Furthermore, the finding that CBP22 recognized only GlcNAc, while CBP70 recognized both GlcNAc and Glc, with a higher affinity for the former, remains unclear. The presence of two distinct CRDs in CBP70, one binding Glc and the other GlcNAc, can be ruled out because all the CBP70 was eluted from Glc and GlcNAc columns with GlcNAc and Glc, respectively. The difference between the two sugars lies merely in the presence or the absence of one acetyl group. One possibility may be that CBP22 can recognize the part of the GlcNAc molecule which contains the acetyl group, whereas CBP70 can bind the other part of the molecule which is identical in Glc and GlcNAc.

Until now, the hypothesis of involvement of glycoprotein-lectin interactions in nuclear activities was essentially supported by the results obtained by different groups using the exogenous lectin WGA. The identification of nuclear GlcNAc-binding proteins, reported here, renders quite plausible the postulate that O-GlcNAc proteins could be potential ligands for these lectins. Thus, the concept that glycoprotein-lectin interactions could play an important role in nuclear activities is strengthened. Experiments are continuing in this field to further confirm the existence of such intranuclear interaction.

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