ELSEVIER

Journal of Chromatography B, 706 (1998) 167–171

JOURNAL OF CHROMATOGRAPHY B

Short communication

Affinity purification and characterization of recombinant human galectin-1

Magali Fouillit[a], Matthieu Lévi-Strauss[b], Virginie Giudicelli[a], Didier Lutomski[a], Dominique Bladier[a], Michel Caron[a,*], Raymonde Joubert-Caron[a]

[a]*Biochimie Cellulaire des Hémopathies Lymphoïdes, UFR SMBH-Léonard de Vinci, Université Paris Nord, 74, rue Marcel Cachin, F-93017 Bobigny, Cédex, France*

[b]*INSERM U 25, Immunologie Clinique, Hôpital Necker, 161 rue de Sèvres, 75743 Paris, Cédex 15, France*

Abstract

Galectin-1, a polypeptidic factor that can have major effects on cell growth and apoptosis, was overexpressed in *E. coli*. This protein was purified to homogeneity by affinity chromatography on lactose coupled to divinylsulfone-activated agarose. The recombinant galectin-1 (rGAL1) was compared with the homologous protein purified from human brain tissue using two-dimensional electrophoresis on immobilized pH gradient (IPG-DALT). rGAL1 had a major isoelectric point of 5.4 (major p*I* of tissular galectin-1, 5.1) and its subunit molecular mass was 14 500. Addition of rGAL1 to Jurkat T-lymphoblastoid cells induced cell death in a concentration-dependent manner. © 1998 Elsevier Science B.V.

Keywords: Polypeptides; Galectin-1

1. Introduction

Galectin-1 (GAL1) is a member of a family of animal carbohydrate-binding proteins and is expressed in some hematopoietic cells [1–3]. It is a dimeric protein [4] with a subunit M_r of 14 500. When added to cells or overexpressed, GAL1 can have major effects on cell growth and apoptosis [2,5,6]. Like other galectin family members, it lacks a signal sequence for import into the endoplasmic reticulum. Nevertheless, it has been shown to be exported from the cytoplasm by a novel mechanism independent of the classical secretary pathway [7–9], and it can even be exported when expressed as a recombinant protein in *Saccharomyces cerevisiae* [10]. Secretion of GAL1 and binding to cell surface receptors are necessary for autocrine extracellular functions.

As a first step to study the link between the binding of GAL1 to the cell surface and the induction of apoptosis in hematopoietic cells, human GAL1 has been expressed as a recombinant protein in *Escherichia coli* and purified by affinity chromatography. Recombinant GAL1 has been compared by two-dimensional electrophoresis with the homologous protein purified from human tissue. The human T-cell line Jurkat was used as a model to study the

*Corresponding author.

0378-4347/98/$19.00
PII S0378-4347(97)00336-8

effect of the purified recombinant protein on cell death.

2. Experimental

2.1. Materials and reagents

Except when otherwise indicated, chemicals and other reagents were obtained from Sigma (La Verpillière, France). The equipments for IEF and horizontal SDS–PAGE (Multiphor II electrophoresis chamber, Immobiline strip tray, Excel gels, Multidrive XL 3500 and EPS-500 power supplies) were from Pharmacia Biotech (Saclay, France). PD-10 columns containing Sephadex G-25 M were from the same company. A Densitometer GS-700, Molecular Analyst software and Melanie II-2D-PAGE release 2.05 for Power Macintosh computers were from Biorad (Ivry-sur-Seine, France).

Human brain GAL1 was purified from soluble brain extract by chromatography on lactosyl–agarose (E.Y. Laboratories, San Mateo, CA, USA) as previously described [11], and it showed apparent homogeneity by SDS–PAGE. Protein concentration was estimated by the Bradford method with the Bio-Rad Protein Assay Reagent.

2.2. Expression of rGAL1

The human GAL1 cDNA was prepared using a template total RNA extracted from human substantia nigra and kindly provided by Dr. H. Chneiweiss (INSERM, U114). Briefly, the reverse transcription of 25 μg of total RNA were primed with oligo-dT_{12-18} and an aliquot of the reaction was amplified by polymerase chain reaction (PCR) (30 cycles: 94°C, 1.5 min; 56°C, 1.5 min; 72°C, 2 min) with primers corresponding to positions 27–46 and 501–482 of the published human cDNA sequence (Genbank: HUMLEC) [12]. The 475-bp long fragment was gel-purified and an aliquot was subjected to another round of amplification using the same downstream primer (501–482) and a mutator upstream primer (GACTCAACCATGGCTTGTGG) creating a *Nco*I restriction site at the level of the initiator ATG. After restriction with *Nco*I (a natural *Nco*I site is located after the stop codon, at position 469 of the published sequence), the 421-bp long fragment was gel-purified and inserted into a *Nco*I-cleaved, dephosphorylated pQE-60 procaryotic expression vector (QUIAGEN, Hilden, Germany). Twelve M15[pREP4] transformants were grown in liquid medium and those expressing human galectin were identified after isopropyl-D-thiogalactoside (IPTG) induction by Western blotting using a polyclonal anti-human GAL1 rabbit antiserum [13]. A positive plasmid was selected and its cDNA insert was sequenced in order to check the absence of mutations modifying the primary structure of the recombinant protein.

2.3. Purification of rGal1

The bacteria were grown in LB medium (Gibco) containing ampicillin (100 μg/ml) and kanamycin (25 μg/ml) at 37°C. After reaching an absorbance of 0.6 units at 650 nm, they were induced to synthesize rGAL1 by IPTG at a concentration of 2 m*M*, for 2–4 h at 37°C. No insoluble rGAL1 was observed upon expression. Cells were then collected by centrifugation (4°C, 14 000 *g*, 20 min), suspended with ice-cold extraction buffer (50 m*M* Tris–HCl, pH 7.4, 10 m*M* EDTA, 4 m*M* β-mercaptoethanol, 0.25 m*M* phenylmethyl sulfonylfluoride (PMSF), 0.1 μ*M* aprotinin, 1 μ*M* pepstatin, 1 μ*M* leupeptin), and sonicated. After sedimentation at 15 000 *g* for 30 min at 4°C, the clear supernantant was collected. The extraction procedure was repeated three times. The protein content of the soluble extracts was determined by the method of Bradford [14]. rGAL1 was purified on a column of lactosyl–agarose [15]. The column was washed with 50 m*M* Tris–HCl, pH 7.4, containing 4 m*M* β-mercaptoethanol, 0.25 m*M* PMSF, 0.02% sodium azide (MTB). The bound rGAL1 was eluted with the same buffer containing 100 m*M* lactose. Fractions containing rGAL1 were dialized against MTB and stored at −20°C.

The purified protein was analyzed by SDS–PAGE under reducing conditions on Phastgels 10–15% (Pharmacia Biotech, Saclay, France) or by two-dimensional PAGE with immobilized pH gradient (IPG-DALT) [3] and visualized by silver staining. Gel imaging was achieved using a laser densitometer (Densitometer GS-700, Biorad).

2.4. Assays for cell growth and viability

Before performing the assay, gel permeation chromatography of rGAL1 was carried out in a Pharmacia PD-10 column equilibrated with sterile/apyrogenic 0.15 *M* NaCl solution. The sample (total volume of 1 ml) was eluted in the same solution, and then sterilized by UV irradiation and used immediately.

Jurkat cells (4×10^5 cells/ml in RPMI 1640 medium containing 1% FCS) were seeded into flat-bottomed 96-well plates (40 000 cells/well) and cultured for 24 h at 37°C. A 50-μl aliquot of rGAL1 (ranging from 400 ng/ml to 40 μg/ml) was added for different times. Viable cell numbers were determined by the MTS assay [16], using a CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay kit (Promega). Cell death was calculated as follows:

$$\%\ \text{cell death} = \frac{A_{490}(\text{cells} + \text{NaCl}) - A_{490}(\text{cells} + \text{rGAL1})}{A_{490}(\text{cells} + \text{NaCl})} \times 100$$

3. Results and discussion

Following extraction and removal of the particulate material by centrifugation, 30 ml of bacteria supernatant were applied at 20°C to a column containing 15 ml of lactosyl–agarose. The column was washed extensively in order to remove weakly bound proteins, and rGAL1 was eluted at 4°C with the washing buffer that contained 100 m*M* lactose. The yield of purified rGAL1 was 13 mg, i.e. approximately 20% of the total soluble proteins of the crude bacteria extract. The purified galectin showed a single polypeptide corresponding to approximately 14.5 kDa on SDS–polyacrylamide gel electrophoresis under reducing conditions (Fig. 1), which is the expected size of GAL1.

As rGAL1 and tissular human GAL1 (GAL1 purified from human brain) were indistinguishable from each other by SDS–PAGE, 2-D electrophoresis was used to confirm or not their complete identity. Under denaturing conditions, the 2-D electrophoresis of both galectins showed spots at M_r 14 500. As reported for most highly purified galectins from

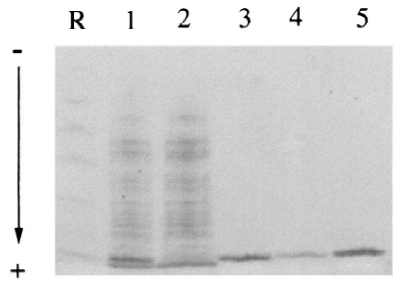

Fig. 1. SDS-PAGE analysis of rGAL1 expressed in *E. coli* and isolated on a lactose–agarose column. Samples from the column were electrophoresed on a 10–15% gradient gel, stained with silver, and scanned using a laser densitometer. Standards shown in lane R are as follows: phosphorylase b, 94 kDa; serum albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30.4 kDa; trypsin inhibitor, 20.1 kDa; α-lactalbumin, 14.4 kDa. Lane 1 *E. coli* extract; lane 2, lactose–agarose column flow-through; lanes 3–5, lactose–agarose column eluate.

diverse sources, the presence of isoform profiles was observed in recombinant and tissular GAL1 preparations. As previously described [3], the tissular GAL1 was resolved in several spots with a prominent one at pH 5.1. The rGAL1 consisted of four spots: a prominent one (80%) at pH 5.40 and three minor spots at 5.05, 5.10, 5.75 (Fig. 2). This range of p*I* values is slightly higher than the one determined for tissular GAL1, and the p*I* value of the major component of rGAL1 is close to the theoretical value (p*I* 5.34) deducted from the primary structure in the Swiss-Prot database [17]. An hypothesis that needs to be confirmed by additional experiments is that the two galectin preparations show different p*I* profiles because the tissular GAL1 is *N*-acetylated [18]. The apparent modifications of the p*I* of proteins leading to apparent isoforms revealed by 2-D gels have been described for various proteins, and the extent of heterogeneity that exists naturally in biological sys-

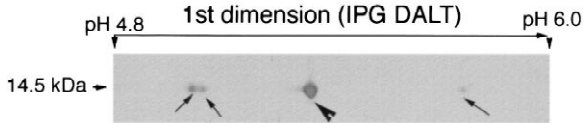

Fig. 2. Two-dimensional (2D)-polyacrylamide gel electrophoresis with immobilized pH gradient: rGAL1 pattern obtained with Immobiline strip pH 4–7 in the first dimension. The 2D pattern is shown as a computer-derived image from silver-stained gel. The arrowhead indicates the position of the prominent spot (pH 5.40) and the three arrows the minor spots at pH 5.05, 5.10, and 5.75.

tems remains poorly defined [19,20]. In some cases, these apparent isoforms are differentially post-translationally modified versions of a single polypeptide. But no post-translational modifications that could provide a basis for the presence of isoforms in GAL1 have been demonstrated until now. An hypothesis is that, as GAL1 contains six cysteine residues, the oxidative state of these different cysteines may influence the focusing of the protein [15].

To determine if affinity-purified rGAL1 could induce cell death, Jurkat T cells were treated with various concentrations of the galectin. rGAL1 was used as a sterile solution in 0.15 *M* NaCl, and controls were performed using sterile NaCl solution instead of galectin. As shown in Fig. 3, rGAL1 induced death of Jurkat cell. The percentage of cell death increased with the concentration of galectin, reaching 55% at the higher concentration assayed. This result reveals that rGAL1 can remain fully active in the absence of reducing agents, in conditions where it is in interaction with cell membrane ligands. Similar observations were obtained with GAL1 from other species in interaction with artificial ligands: the toad ovary GAL1 stored in the presence of soluble or solid-phase-bound ligand [21] and the CHO GAL1 when bound to laminin-Sepharose [22].

Summarizing our results, a simple method has been proposed permitting to obtain a powerful tool for the study of GAL1-induced cellular events. Stored at −20°C in β-mercaptoethanol-containing buffer, rGAL1 samples remain stable for several months, and a rapid buffer exchange allows to obtain a protein solution that can be used immediately for in vitro biological tests.

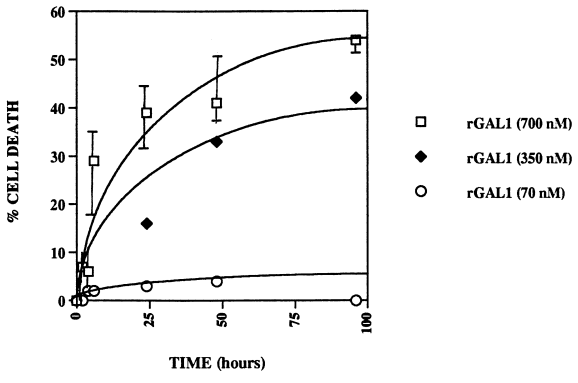

Fig. 3. Affinity-purified rGAL1 induces cell death in Jurkat cells. Viable cell numbers were determined by the MTS assay. Each point represents the mean from three cultures. Data of cells incubated with 700 n*M* rGAL1 are arithmetic means±S.D.

Acknowledgments

The authors thank Ms. Françoise Arnos for sequencing of the recombinant plasmids. This work was supported by grants form the Direction de la Recherche et des Etudes Doctorales (DRED), from the Association pour la Recherche contre le Cancer (Grant 1720), and from the Ligue Française contre le Cancer (Comité de la Seine Saint-Denis). D.L. is a recipient from the Association pour la Recherche contre le Cancer.

References

[1] S. Gabius, K.P. Hellmann, T. Ciesiolka, N.A. Nagel, H.J. Gabius, Blut 59 (1989) 165.
[2] S.D. Goldstone, M.F. Lavin, Biochem. Biophys. Res. Commun. 178 (1991) 746.
[3] D. Lutomski, M. Caron, J.D. Cornillot, P. Bourin, C. Dupuy, M. Pontet, D. Bladier, R. Joubert-Caron, Electrophoresis 17 (1996) 600.
[4] V. Guidicelli, D. Lutomski, M. Lévi-Strauss, D. Bladier, R. Joubert-Caron, M. Caron, Glycobiology 7 (1997) VIII.
[5] N.L. Perillo, K.E. Pace, J.J. Seilhamer, L.G. Baum, Nature 378 (1995) 736.
[6] V. Wells, L. Mallucci, Cell 64 (1991) 91.
[7] D.N.W. Cooper, S.H. Barondes, J. Cell Biol. 110 (1990) 1681.
[8] R. Lindstedt, G. Apodaca, S.H. Barondes, K.E. Mostov, H. Leffler, J. Biol. Chem. 268 (1993) 11750.
[9] V. Avellana-Adalid, G. Rebel, M. Caron, J.D. Cornillot, D. Bladier, R. Joubert-Caron, Glycoconjugate J. 11 (1994) 286.
[10] A.E. Cleves, D.N.W. Cooper, S.H. Barondes, R.B. Kelly, J. Cell Biol. 133 (1996) 1017.
[11] V. Avellana-Adalid, R. Joubert, D. Bladier, M. Caron, Anal. Biochem. 190 (1990) 26.
[12] P.O. Couraud, D. Casentini-Borocz, T.S. Bringman, J. Griffith, M. McGrogan, G.E. Nedwin, J. Biol. Chem. 264 (1989) 1310.
[13] D. Bladier, R. Joubert, V. Avellana-Adalid, J.L. Kémény, C. Doinel, J. Amouroux, M. Caron, Arch. Biochem. Biophys. 269 (1989) 433.
[14] M.M. Bradford, Anal. Biochem. 72 (1976) 248.
[15] V. Avellana-Adalid, R. Joubert-Caron, M. Caron, D. Bladier, Electrophoresis 13 (1992) 416.
[16] T.M. Buttke, J.A. McCubrey, T.C. Owen, J. Immunol. Methods 157 (1993) 233.

[17] R.D. Appel, A. Bairoch, D.F. Hochstrasser, Trends Biochem. Sci. 19 (1994) 258.
[18] M. Caron, D. Bladier, R. Joubert, Int. J. Biochem. 22 (1990) 1379.
[19] C. Damerval, M. Zivy, F. Granier, D. De Vienne, Adv. Electrophoresis 2 (1988) 236.
[20] M.R. Wilkins, J.C. Sanchez, K.L. Williams, D.F. Hochstrasser, Electrophoresis 17 (1996) 830.
[21] H. Ahmed, J. Pohl, N.E. Fink, F. Strobel, G.R. Vasta, J. Biol. Chem. 271 (1996) 33083.
[22] M. Cho, R.D. Cummings, J. Biol. Chem. 270 (1995) 5198.