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Study of lectin–ganglioside interactions by high-performance liquid affinity chromatography

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ABSTRACT

A high-performance affinity column containing immobilized modified GM1 (lyso-GM1) was used to study the binding of an endogenous human brain lectin (HBL) in comparison with other carbohydrate-binding proteins. The proteins are previously converted into biotinylated derivatives. Detection of biotinylated proteins in the eluates by a microtitre plate assay ensures good sensitivity. The maximum binding capacity of the adsorbent for HBL is obtained in Tris buffer supplemented with β-mercaptoethanol. The binding is inhibitable by specific sugar. It is concluded that the use of immobilized glycolipids in analytical high-performance liquid affinity chromatographic methods may serve as models in the study of interactions between gangliosides and carbohydrate-binding proteins.

INTRODUCTION

Surface gangliosides, like membrane glycoproteins, are believed to play a major role in cell recognition and cell to cell interactions [1–3]. Gangliosides can serve as selective receptor sites for several bioeffectors such as neurotransmitters, carbohydrate-binding proteins (lectins) and toxins [4–6]. The interaction between ganglioside GM1 [Gal β1–3GalNAcβ1–4(NeuAc α2–3)Gal β1–4Glcβ1-Cer] and cholera toxin for example, has been studied in great detail [4,5,7–10].

Although the molecules which recognize these gangliosides *in vivo* are certainly numerous, evidence suggests that complementary carbohydrate-binding proteins may be involved. Previous work has demonstrated the specific recognition by plant lectins of liposomes containing gangliosides and of poly(vinyl chloride)-adsorbed gangliosides [11–13]. The results of these studies clearly depended on the assay system used and on the experimental conditions. Another approach has been to examine the ability of synthetic saccharide derivatives, which can be considered as neoglycolipids [14,15], to interact with

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endogenous lectins. In spite of this, however, little is known about the possible interactions between gangliosides and carbohydrate-binding proteins which can co-exist in the same organ, and particularly in brain tissue where glycosphingolipids are particularly abundant.

For many years, we have examined one brain tissue lectin that we particularly characterized in human [16,17]. Hapten inhibition studies and binding of blood group-related synthetic oligosaccharides showed that it is actually specific for structures containing lactose or lactosamine, such as blood group i antigen. However, its possible interaction with brain gangliosides remains to be clarified. To elucidate this aspect, we have now used an analytical high-performance liquid affinity chromatographic (HPLAC) method, based on porous silica beads derivatized with GM1. In addition, some comparisons of the binding activity of the human brain protein with those of plant lectins and cholera toxin are presented.

EXPERIMENTAL

Proteins and derivatives

The main characteristics of the carbohydrate-binding proteins used in this work are summarized in Table I.

HBL was purified as a carboxamidomethylated derivative in a bioactive form as described previously [16,18].

The lectin from *Arachis hypogaea* (peanut agglutinin, PNA) was purified by the Laboratoire de Biochimie et Technologie des Protéines. The B subunit of cholera toxin (CT-B) and the lectins from *Triticum vulgaris* (wheat germ agglutinin, WGA) and edible snail (*Helix pomatia* agglutinin, HPA) were obtained from Sigma Chimie (La Verpillière, France). All the carbohydrate-binding proteins were biotinylated according to Avellana-Adalid *et al.* [18].

High-performance liquid affinity chromatography (HPLAC) on Spherosil–DEAE-dextran beads derivatized with GM1

Spherosil beads coated with DEAE-dextran and coupled with lyso-GM1 were prepared as described previously [19,20]. They were packed in a glass column (50 × 5 mm I.D.) of bed volume 1 ml.

Chromatographic experiments were carried out using a high-performance liquid chromatographic (HPLC) system (LKB) consisting of high-precision pump (LKB 2249). Routinely, the concentration of each protein used in a sample was 0.25 mg/ml dissolved in the equilibration buffer. The volume injected was 20 μl and the flow-rate was 0.4 ml/min during the 5 first minutes, then, 0.6 ml/min throughout. The elution of adsorbed proteins was performed using a linear gradient from 0 to 100% elution buffer 1 (50 m*M* citrate buffer, pH 2.8; 4 min)

TABLE I

CARBOHYDRATE-BINDING PROTEINS USED IN HPLAC EXPERIMENTS

Origin	Common name	M_r	Major sugar specification[a]
Human brain	HBL	14 500 × 2	Galβ1–4GlcNac > Galβ1–4Glc
Vibrio cholerae	CT-B	11 800	Galβ1–3GalNacβ1–4 (NeuNacα2–3)Gal > GalNac β1-4(NeuNacα2–3)Gal
Triticum vulgaris	WGA	21 600 × 2	$(GlcNac\beta1–4)_3$ > NeuNac
Arachis hypogaea	PNA	24 500 × 4	Galβ1–3GalNAc > Gal
Edible snail	HPA	13 000 × 6	GalNac, GlcNac

[a] Gal = D-galactose; Glc = D-glucose; GalNac = N-acetyl-D-galactosamine; GlcNac = N-acetyl-D-glucosamine; NeuNac = N-acetylneuraminic acid.

followed by 100% elution buffer over 12 min. In some experiments, the bound proteins were eluted by sequential applications of 50 m*M* Tris–HCl buffer (pH 7.6) containing 0.1 *M* lactose (elution buffer 2) and of acidic elution buffer 1.

Four different equilibration buffers tested: (1) 50 m*M* citrate buffer–100 m*M* NaCl (pH 7.2); (2) 50 m*M* citrate buffer–100 m*M* NaCl (pH 4.9); (3) 50 m*M* Tris–HCl buffer–100 m*M* NaCl, 0.4 m*M* β-mercaptoethanol (pH 7.6); and (4) buffer 3 containing 40 m*M* $CaCl_2$.

NaCl was used to suppress ionic adsorption of proteins on the DEAE-dextran monolayer.

Microtitre plate assay

The amount of biotinylated protein in each eluted fraction was determined with streptavidin coupled to horseradish peroxidase (Strep-HRP, Sigma). Microtitre plates (96 wells; Nunc-Immuno Plate Maxisorp, Denmark) were coated with 50 μl of each effuent sample overnight at 4°C. The wells were washed with 10 m*M* potassium phosphate (pH 7.4), containing 0.05% Tween 20, then blocked with 100 m*M* potassium phosphate (pH 7.4) containing 3% bovine serumalbumin (BSA) and 0.3% Tween 20 for 90 min followed by three further washes. The amount of bound protein was detected by using Strep-HRP as described [21].

RESULTS AND DISCUSSION

As detailed above, we developed a protocol that allows the rapid evaluation of the adsorption of carbohydrate-binding proteins on immobilized gangliosides. This technique used silica beads derivatized with lyso-GM1 ganglioside, a chromatographic support originally developed for the purification of cholera toxin on an industrial scale (1 kg column) [20]. We selected CT-B as the reference protein because it is responsible for binding the toxin to GM1 ganglioside receptors. The binding and the subsequent release of the protein from the HPLAC column were studied by microtitre plate assay detection of the effluent. The use of an HPLC system allowed highly reproducible experimental conditions to be obtained and only analytical amounts (20 μl) of carbohydrate-binding protein to be injected.

Binding of biotinylated cholera toxin B subunit to immobilized lyso GM1

The characteristic binding profile of CT-B to the sorbent is shown in Fig. 1. When the biotinylated derivative prepared from this

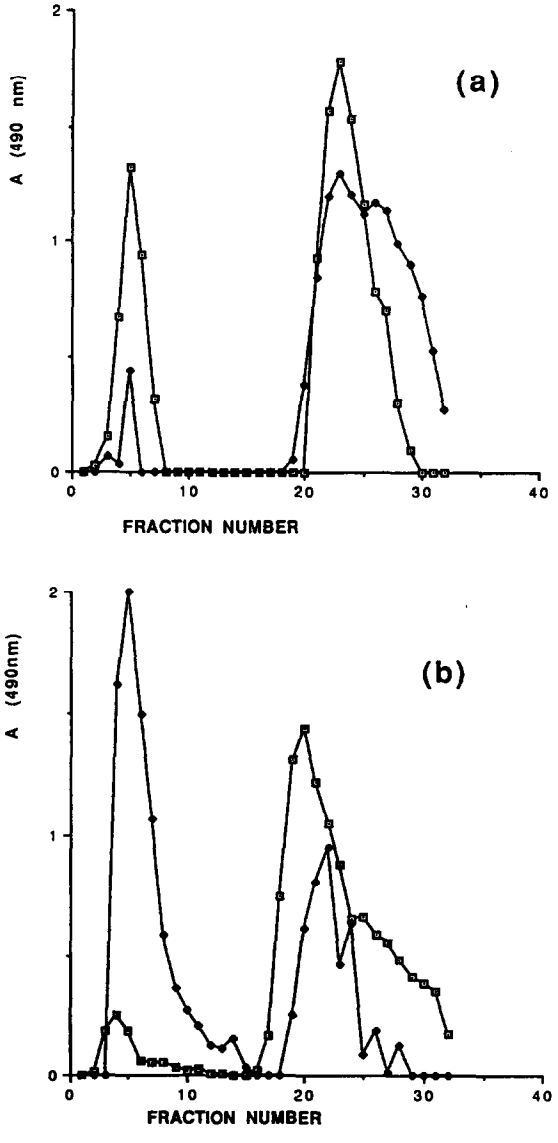

Fig. 1. Chromatograms showing the effect of the equilibration buffer on Spherosil–DEAE-dextran–lyso-GM1 affinity chromatography of cholera toxin B subunit. (a) 50 m*M* citrate buffer [pH 7.2 (buffer 1) (□) and pH 4.9 (buffer 2) (◆)]; (b) 50 m*M* Tris–HCl buffer (pH 7.6) containing β-mercaptoethanol (buffer 3) (□) and $CaCl_2$ (buffer 4) (◆). Sample, 5 μg of protein in equilibration buffer; column, 50 mm × 5 mm I.D.

subunit was passed through the column in a buffer used on the preparative scale (equilibration buffer 1), *ca.* 78% of this protein was bound to the column (Table II). This is in accordance with the fact that CT-B acts as a biologically inactive structural analogue of cholera toxin by virtue of competition for binding to GM1 [22]. This binding increased to values close to 100%using a more acidic buffer or a Tris–HCl buffer containing β-mercaptoethanol. Conversely, it was significantly decreased in the presence of divalent cations. We interpreted these data by the interactions between Ca^{2+} and the negatively charged saccharidic part of the gangliosides.

Binding of biotinylated human brain lectin

HBL-Biot, when analysed by HPLAC, showed no binding in equilibration buffer 1 (Fig. 2, Table II). In contrast, a significant percentage of the total protein was able to recognize the immobilized ganglioside in more reducing conditions (acidic pH or in the presence of β-mercaptoethanol). These results confirm the thiol dependence of HBL and, more generally, of the lectins belonging to the same protein family [23]. According to the saccharidic specificity of both the lectin and its biotinylated derivative [16–18], one may suggest that the interaction occurs through the internal lactosyl of the ganglioside. The fact that an unbound protein fraction was always found in the effluent indicates either that the lectin was bound with a relatively low affinity or that different conformational forms of the lectin present different affinities for the lyso-

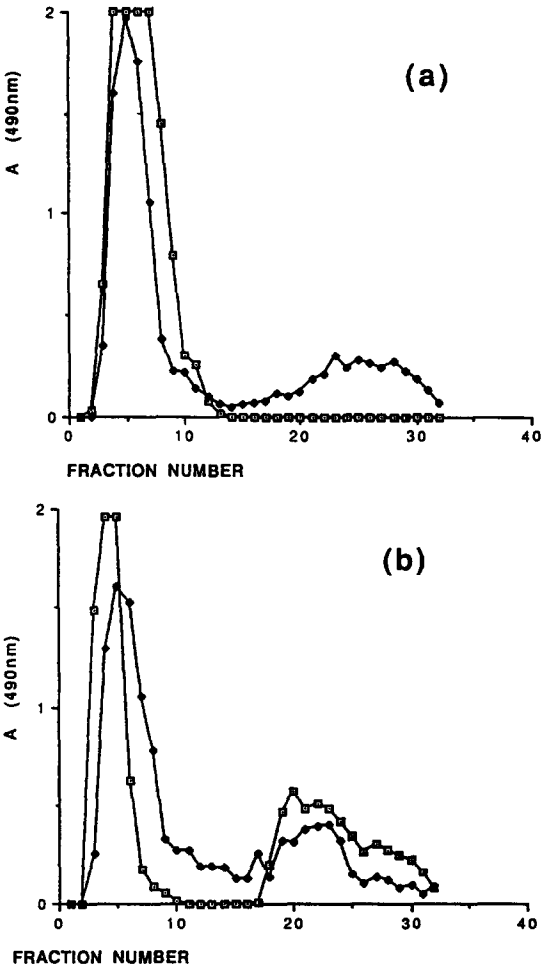

Fig. 2. Chromatograms showing the effect of the equilibration buffer on Spherosil–DEAE-dextran–lyso-GM1 affinity chromatography of human brain lectin and symbols as in Fig. 1.

TABLE II

RECOVERIES (%) OF RETAINED–ELUTED PROTEINS

Equilibration buffer	CT-B	HBL	WGA	PNA	HPA
(1) Citrate buffer (pH 7.2)	77.7	0	47.0	87.7	61.7
(2) Citrate buffer (pH 4.9)	98.8	28.2	24.9	95.6	62.5
(3) Tris buffer containing β-mercaptoethanol	92.6	43.7	41.6	43.2	48.3
(4) Tris buffer containing β-mercaptoethanol and Ca^{2+}	33.9	28.1	5.4	49.7	28.9

GM1 immobilized on the affinity support. Such a microheterogeneity has been reported for HBL. It may be the result of pH-dependent conformational changes, leading to a panel of differently charged molecular species [24].

To test these expectations, the void volume peaks obtained in buffer 3 for CT-B and HBL were rechromatographied. A separation into two peaks was obtained for both proteins in proportions close to those obtained during the first chromatography. These results suggest that, even if the presence of molecular forms showing different affinities cannot be completely excluded, the chromatographic profiles certainly reflect the affinity of the whole protein populations.

Binding profiles of a panel of carbohydrate-binding proteins

We used the protocol established with TC-B and HBL to determine the binding profile of several lectins which are specific for saccharidic constituents of the gangliosides. Table II lists the binding capacities obtained.

Of the proteins, WGA is known to bind to GM1-bearing liposomes [11], certainly by the mediation of N-acetylneuraminic acid, whereas poly(vinyl chloride)-adsorbed GM1 will not bind the lectin [13]. WGA bound only moderably to the affinity support. In particular, a comparison of the relative binding capacities in neutral and acidic citrate buffer showed that among the carbohydrate-binding proteins tested only WGA showed a pH-dependent affinity which could be related to the dissociation of the protein into monomers in acidic media.

Using poly(vinyl chloride)-adsorbed glycosphingolipids, Molin *et al.* [13] reported that PNA showed the highest afinity to asialo-GM1, but also bound, though less strongly, to GM1, whereas Momoi *et al.* [12] found no binding of GM1 to the lectin. Although silica beads derivatized with GM1 bound PNA under a variety of conditions, including Ca^{2+}-containing buffer, the highest binding capacities were obtainable when the sample was applied in citrate buffers. This is especially true for the binding at pH 4.9. As a consequence, it is not necessary to subject the ganglioside to a desialylation prior to obtain a binding of the lectin under these experimental conditions. However, the affinity and capacity of GM1 for PNA are clearly limited compared with more specic adsorbents such as beads derivatized with lactose.

HPA has been used for the detection of glycolipids with terminal GalNAc residues on thin-layer chromatograms and for the affinity purification of the corresponding glycolipid-derived oligosaccharides [25]. Despite its specificity for GalNAc end-groups, the snail lectin is partially adsorbed on the affinity matrix. Although it has a very low affinity for galactose, one may assume that the large number of galactosyl end-groups must provide sufficient binding loci to cause the lectin to be adsorbed. The same kind of hypothesis has been proposed to explain the fact that HPA can be purified by adsorption to Sephadex followed by elution with galactose or glucose [26]. Another possibility would be the hydrolysis of a small number of Galβ(1–3) GalNAc linkages leading to some high-affinity sites for the lectin.

Sequential elutions of HBL and cholera toxin

With a view to ascertaining the carbohydrate specificity of the interaction between brain lectin and immobilized GM1, the bound protein was eluted by sequential application of buffer containing 0.1 *M* lactose and citrate buffer (pH 2.8). An elution peak containing about 82% of the adsorbed lectin was obtained with the 0.1 *M* lactose solution. That the recognition of GM1 by HBL depends on a different mechanism to the well documented interaction of the same ganglioside with cholera toxin was verified using TC-B instead of the lectin. No elution was obtained with lactose, in accord with the observation that lactosylceramide does not inhibit the binding of cholera toxin to membrane gangliosides [10]. Fig. 3 compares the chromatograms obtained with the two proteins.

CONCLUSIONS

The results reported here established the feasibility of studying protein–glycolipid interactions by use of simple HPLAC procedures. The binding of specific lectins to oligosaccharide

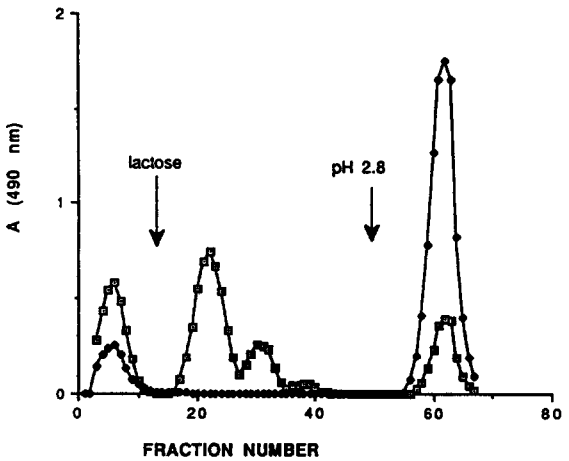

Fig. 3. Chromatogram showing the elution of (□) HBL and (●) cholera toxin subunit B adsorbed on lyso-GM1. Successive elutions were performed (arrows) with Tris–HCl buffer containing 0.1 *M* lactose and citrate buffer (pH 2.8).

affinity columns was previously used as a relatively simple system to define their carbohydrate specificity [14, 27]. However, protein–glycolipid interactions can be more complex, involving contributions of oligosaccharide and ceramide portions of the ganglioside [10]. We used HPLAC to detect a possible recognition phenomenon between two molecules that co-exist in brain tissue, a soluble lectin and the monosialoganglioside GM1. The binding capacity of the brain protein was compared with those of other carbohydrate-binding proteins, including the well known biological partner of the glycolipid, cholera toxin. All the experiments were conducted in buffers containing a concentration of NaCl previously described to avoid the affinity support to work as an ion-exchange matrix [20].

We can conclude that HBL is able to interact with GM1. However, only half of the molecules were adsorbed during the chromatography. A possible explanation of this result is that it reflects a low affinity between the two partners. This low affinity could be due either to a repulsive effect of the sialic acid or to an inhibitory effect of NaCl [28]. It is interesting that (i) this binding was obtained only in a buffer used during other methods to preserve the activity of the lectin (except NaCl concentration) and (ii) addition of lactose to the buffer is sufficient to eluate the lectin. It is consistent with the idea that the reaction between HBL and GM1 is carbohydrate specific.

Another point is the differences observed between the results obtained in HPLAC and in other assays. This is not completely surprising as the influence of the experimental conditions during these interactions is well established. An advantage of the HPLAC approach is that it allows this influence to be studied by modifying the composition of the buffers. Further, one may suggest that the interaction of proteins showing a moderate affinity for the ganglioside will be easier than in other assays. A possible explanation is that when the GM1 is present at the end of a spacer arm on an affinity matrix it may be able to rotate more freely and assume a configuration and an orientation that the proteins can recognize.

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REFERENCES

1 S.I. Hakomori, *Rev. Biochem.*, 50 (1981) 733.
2 S. Ando, *Neurochem. Int.*, 5 (1983) 507.
3 R. D. Dal Toso, S.D. Skaper, G. Ferrari, G. Vantini, G. Toffano and A. Lear, in D.G. Stein and B.A. Sabel (Editors), *Pharmacological Approaches to the Treatment of Brain and Spinal Cord Injury*, Plenum Press, New York, 1988, pp. 143–164.
4 W.E. Van Heyningen, *Nature*, 249 (1974) 415.
5 P. Cuatrecasas, *Biochemistry*, 12 (1973) 3558.
6 C.C. Blackburn, P. Swank-Hill and R.L. Schnaar, *J. Biol. Chem.*, 261 (1986) 2873.
7 P. Cuatrecasas, I. Parikh and M.D. Hollenberg, *Biochemistry*, 12 (1973) 4253.
8 C.L. Schengrund and N.J. Ringler, *J. Biol. Chem.*, 264 (1989) 13233.

9 K.C. Joseph, A. Stieber and N.K. Gonatas, *J. Cell. Biol.*, 81 (1979) 543.
10 P. Cuatrecasas, *Biochemistry*, 12 (1973) 3547.
11 D.H. Boldt, S.F. Speckart, R.L. Richards and C.R. Alving, *Biochem. Biophys. Res. Commun.*, 74 (1977) 208.
12 T. Momoi, T. Tokunaga and Y. Nagai, *FEBS Lett.*, 141 (1982) 6.
13 K. Molin, P. Fredman and L. Svennerholm, *FEBS Lett.*, 205 (1986) 51.
14 J.C. Solomon, M.S. Stoll, P. Penfold, W.M. Abbott, R.A. Childs, P. Hanfland and T. Feizi, *Carbohydr. Res.*, 213 (1991) 293.
15 A. Chadli, M. Caron, M. Ticha, R. Joubert, D. Bladier and J. Kocourek, *Anal. Biochem.*, 204 (1992) 198.
16 D. Bladier, R. Joubert, V. Avellana-Adalid, J.L. Kemeny, C. Doinel, J. Amouroux and M. Caron, *Arch. Biochem. Biophys.*, 269 (1989) 433.
17 D. Bladier, J.P. LeCaer, R. Joubert, M. Caron and J. Rossier, *Neurochem. Int.*, 18 (1991) 275.
18 V. Avellana-Adalid, R. Joubert, D. Bladier and M. Caron, *Anal. Biochem.*, 190 (1990) 26.
19 J.L. Tayot and M. Tardy, in L. Svennerholm, H. Dreyfus and P.F. Urban (Editors), *Structure and Function of Gangliosides*, Plenum Press, New York, 1980, pp. 471–478.
20 J.L. Tayot, J. Holmgren, L. Svennerholm, M. Lunblad and M. Tardy, *Eur. J. Biochem.*, 113 (1981) 249.
21 H. Eloumami, M. Caron, R. Joubert, C. Doinel and D. Bladier, *J. Neurol. Sci.*, 105 (1991) 6.
22 P. Cuatrecasas, *Biochemistry*, 12 (1973) 3577.
23 M. Caron, D. Bladier and R. Joubert, *Int. J. Biochem.*, 22 (1990) 1379.
24 V. Avellana-Adalid, R. Joubert-Caron, M. Caron and D. Bladier, *Electrophoresis*, 3 (1992) 416.
25 B.V. Torres, D.K. McCrumb and D.F. Smith *Arch. Biochem. Biophys.*, 262 (1988) 1.
26 I.J. Goldstein and C.E. Hayes, *Adv. Carbohydr. Chem. Biochem.*, 35 (1978) 127.
27 H.J. Gabius, *Anal. Biochem.*, 189 (1990) 91.
28 N.E. Fink de Cabutti, M. Caron, R. Joubert, M.T. Elola, D. Bladier and J. Herkovitz, *FEBS Lett.*, 223 (1987) 330.