Carbohydrate Binding Activities of *Bradyrhizobium japonicum*. II. Isolation and Characterization of a Galactose-specific Lectin

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Abstract. Extracts of *Bradyrhizobium japonicum* were fractionated on Sepharose columns covalently derivatized with lactose. Elution of the material that was specifically bound to the affinity column with lactose yielded a protein of M_r ~38,000. Isoelectric focusing of this sample yielded two spots with pI values of 6.4 and 6.8. This protein specifically bound to galactose-containing glycoconjugates, but did not bind either to glucose or mannose. Derivatives of galactose at the C-2 position showed much weaker binding; there was an 18-fold difference in the relative binding affinities of galactose versus *N*-acetyl-D-galactosamine. These results indicate that we have purified a newly identified carbohydrate-binding protein from *Bradyrhizobium japonicum*, that can exquisitely distinguish galactose from its derivatives at the C-2 position.

IN previous studies (5), as well as in the accompanying manuscript (7), we had documented that *Bradyrhizobium japonicum* exhibits four saccharide-specific binding activities: (*a*) adsorption to Sepharose beads derivatized with lactose (Lac);[1] (*b*) homotypic autoagglutination; (*c*) heterotypic binding to cultured soybean (SB-1) cells; and (*d*) heterotypic adhesion to soybean roots. In all four of these assays, galactose (Gal) inhibited the binding, but a C-2 derivative of the monosaccharide, *N*-acetyl-D-galactosamine (GalNAc) failed to yield the same effect. Mutants of *B. japonicum*, isolated on the basis of a defect in one binding activity (SB-1 cell binding), showed a concomitant loss in the other three binding capacities (7). These observations suggested that all of these processes may be mediated by the same component(s) and mechanism(s). We, therefore, began to search for Gal-specific carbohydrate-binding proteins from *B. japonicum*, particularly one that can distinguish Gal from GalNAc.

In the present communication, we report the purification and characterization of a carbohydrate-binding protein, isolated from *B. japonicum* on the basis of its binding to Sepharose columns derivatized with the disaccharide lactose and specific elution with either Gal or Lac. Analysis of the binding specificity of this protein indicated that it can distinguish Gal from its C-2 derivatives. We also document that little or no lectin activity could be found in mutants of *B. japonicum* that have defective carbohydrate-specific binding activities.

Materials and Methods

Isolation of Bradyrhizobium Lectin BJ38

Bradyrhizobium japonicum (R110d) was obtained from the late Dr. Barry

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1. *Abbreviations used in this paper:* BJ38, *Bradyrhizobium japonicum* galactose-binding protein (M_r ~38,000); Gal, galactose; GalNAc, *N*-acetyl-D-galactosamine; Lac, lactose.

Chelm of Michigan State University and was cultured in yeast extract-mannitol-gluconate medium as previously described (6). A 50-ml culture of the bacteria at stationary phase was inoculated into 2 liters of yeast extract-mannitol-gluconate medium and cultured for 2 d. Aliquots of 400 ml of this culture were then inoculated into another 2 liters of medium. The bacteria were cultured for 24 h at 30°C on a gyratory shaker set at 100 rpm. Bacteria were harvested by centrifugation for 15 min in a Sorvall GS3 rotor at 8,000 rpm.

The collected bacteria were frozen at −20°C overnight, thawed, and then resuspended in 150 ml of PBS (10 mM sodium phosphate, 0.14 M NaCl, 4 mM KCl, pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride. The sample was subjected to two passages through the French press at 1,600 psi at 4°C. Triton X-100 was then added to a final concentration of 1% (vol/vol). The bacteria were extracted for 30 min at room temperature. The inclusion of detergent in the extraction buffer was intended to assure complete extraction of the lectin. After extraction, the lectin tends to be soluble even without detergent. The extract was centrifuged at 15,000 rpm in a Sorvall SS-34 rotor for 30 min. The supernatant was subjected to affinity chromatography on Sepharose covalently derivatized with lactose (Lac-Sepharose) at room temperature.

Lactose was coupled to epichlorohydrin-activated Sepharose 4B beads according to the procedure developed by Matsumoto et al. (13). For affinity chromatography, a column (1.5 × 7 cm) of Lac-Sepharose was equilibrated with PBS before the supernatant containing the bacterial extract was applied. The column was washed with 400 ml of PBS. Material bound to the column was then eluted with 50 ml of 0.1 M Lac in PBS. Fractions collected after the addition of Lac were concentrated on a filter (Amicon Corp., Danvers, MA) with PM10 membrane.

Radioiodination

Radioiodination was performed by the chloramine T method (11). Samples of BJ38 (100 μl) in PBS containing 0.1 M Lac were added to 1 mCi Na^{125}I. Chloramine T (1 mg/ml) in 25 μl was added to start the reaction. After 1 min, 50 μl of sodium metabisulfite (1 mg/ml) in PBS was added to stop the reaction. The sample was applied to an AG1 × 8 ion-exchange column (0.6 × 3.5 cm; Bio-Rad Laboratories, Richmond, CA) to remove free ^{125}I and Sephadex G-25 column (2.5 × 11 cm) to remove the Lac. The radioactive peak at the void volume of the Sephadex G-25 column was then subjected to affinity chromatography on Lac-Sepharose. The radioactive Lac-eluted fractions were then pooled and 1 ml of 5% BSA solution was added as carrier protein and then dialyzed against PBS to remove the Lac. This sample is designated ^{125}I-BJ38.

Binding of BJ38 to Lac-Sepharose Beads

Lac-Sepharose beads were suspended in PBS at a ratio of 1:1 (vol/vol). Aliquots (0.2 ml) of this bead suspension were transferred to individual culture tubes (12 × 75 mm). Various reagents (in 0.1 ml total volume) were added, followed by 0.1 ml of ^{125}I-BJ38 (1.5 × 10^4 cpm) in PBS. The samples were incubated with gentle shaking at room temperature for 4 h. The samples were then washed three times with 2 ml PBS by centrifugation and resuspension. The amount of radioactivity associated with the beads was then determined. Each binding assay was performed in triplicate.

To investigate the effect of pH on the binding of ^{125}I-BJ38 to Lac-Sepharose beads, the following buffers, each containing 0.15 M NaCl, were used: 10 mM sodium succinate for pH 3.0–6.0; 10 mM phosphate for pH 6.5–7.5; and 10 mM sodium bicarbonate for pH 8.0–11.0. Aliquots of 0.2 ml of the Lac-Sepharose beads (1:1 [vol/vol] in H$_2$O) were added to 0.1 ml of various buffers, followed by 0.1 ml ^{125}I-BJ38 (1.5 × 10^4 cpm). The samples were then incubated for 4 h at room temperature. The beads were centrifuged and the supernatant fractions were collected for pH determination. The beads were then washed three times with 2 ml of 0.15 M NaCl and the amount of radioactivity associated with the beads was determined.

Determinations of the Molecular Weight of BJ38

Protein samples were subjected to gel filtration on a Sephadex G-100 column (1.5 × 114 cm) equilibrated with PBS containing 0.1 M Lac. Fractions of 2 ml were collected. The column was calibrated with blue dextran, BSA, ovalbumin, and chymotrypsinogen as standards.

SDS-PAGE was performed in 10% acrylamide gels as described by Laemmli (10). Two-dimensional PAGE was performed according to O'Farrell's procedure (18) using 2% ampholyte generating a pH gradient from 4 to 8. After electrophoresis, the proteins were revealed by silver staining (14) or by autoradiography using Kodak XAR-5 film.

Test for Lectin Activity in Mutant Bacteria

Two mutants, designated N4 and N6, were derived from *B. japonicum* by chemical mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, followed by selection on the basis of defective binding to cultured SB-1 cells (7). Corresponding 12-liter cultures of wild-type and mutant bacteria were extracted for the isolation of BJ38. After the first cycle of affinity chromatography on Lac-Sepharose columns (1.5 × 7 cm), the bound and Lac-eluted material was iodinated and subjected to a second cycle of Lac-Sepharose affinity chromatography. The level of ^{125}I radioactivity bound to the second Lac-Sepharose column and eluted with Lac was compared between extracts derived from parental and mutant strains of *B. japonicum*.

Results

Carbohydrate-binding Protein from Bradyrhizobium japonicum

Cultures of *B. japonicum* were grown to late logarithmic phase. The cells were ruptured and fractionated by affinity chromatography on a column of Lac-Sepharose. The majority of the protein components, monitored by absorbance at 280 nm, was not bound by the column (Fig. 1, component A). After extensive washing, proteins bound to the column were eluted with 50 ml of 0.1 M Lac in PBS (Fig. 1, component B). Although the 280-nm absorbance profile after Lac addition did not show a distinct peak of protein eluting from the column, there were reproducible and specific proteins bound to the column that were released by the saccharide addition. This conclusion is derived from: (*a*) SDS-PAGE analysis of the material contained in fractions corresponding to component B; and (*b*) radioiodination of component B and rechromatography on a second Lac-Sepharose affinity column (see below).

SDS-PAGE analysis was carried out on component B and compared to the original bacterial extract, as well as to the material not bound to the Lac-Sepharose affinity column
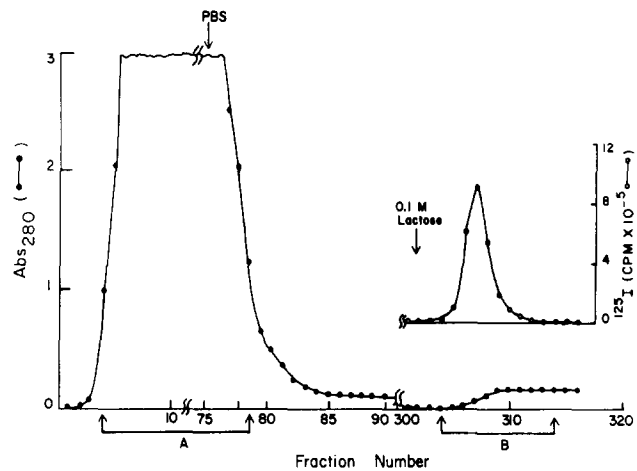

Figure 1. Fractionation of *Bradyrhizobium japonicum* extract on Lac-Sepharose. Extract derived from 12 liters of bacteria was applied to the Lac-Sepharose column (1.5 × 7 cm) equilibrated with PBS. The column was washed with PBS and eluted with 0.1 M Lac (*arrow*). Fractions of 2 ml were collected. The fractions were pooled as indicated in figure and designated as component A and component B. (*Inset*) Component B was then concentrated by ultrafiltration (PM10 membrane) and radioiodinated as described in Materials and Methods. The radioactive sample was then reapplied to the Lac-Sepharose column. The column was washed and eluted with 0.1 M Lac. Radioactivity of the Lac-eluted fractions was determined (●).

(component A). On silver staining of the gel, component B yielded one predominant polypeptide (M_r ∼38,000) plus additional bands (M_r ∼50,000; M_r ∼58,000; M_r ∼62,000; M_r ∼65,000) (Fig. 2, lane *c*). The material not bound by the affinity column (component A) showed a heterogeneous mixture of polypeptides (Fig. 2, lane *b*), as did the original extract (Fig. 2, lane *a*).

Molecular Mass and Isoelectric Points of Lectin BJ38

Component B can be further purified by another cycle of affinity chromatography. The pooled material, accumulated from isolation of 72 liters of bacterial culture, was concentrated and passed over a Sephadex G-25 column to remove the Lac. This material was then subjected to a second cycle of Lac-Sepharose affinity chromatography. SDS-PAGE analysis of the bound and Lac-eluted material from this second affinity column yielded a single polypeptide, as revealed by silver staining of the gel (Fig. 2, lanes *d* and *e*). This polypeptide migrated to a position corresponding to the predominant band observed in the SDS-PAGE of the partially purified material after a single cycle of Lac-Sepharose chromatography (Fig. 2, lane *c*). Identical results were obtained when the electrophoresis was carried out both in the presence (Fig. 2, lane *e*) and the absence (Fig. 2, lane *d*) of reducing agents.

The material corresponding to this polypeptide band (Fig. 2, lane *d*) will be designated hereafter as BJ38 (a M_r ∼38,000 polypeptide from *Bradyrhizobium japonicum*). On two-dimensional gel electrophoretic analysis, highly purified BJ38 yielded two spots, corresponding to M_r ∼38,000 with pI values of 6.4 and 6.8 (data not shown). The intensity of

a b c d e f
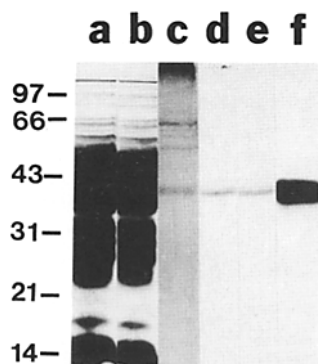
97—
66—
43—
31—
21—
14—

Figure 2. SDS-PAGE analysis of *Bradyrhizobium japonicum* components before and after fractionation on Lac-Sepharose affinity columns. The acrylamide concentration in the gels was 10%. Lanes: (*a*) Total bacterial extract (25 µg); (*b*) component A (25 µg); (*c*) component B was obtained from 24 liters of bacterial culture and concentrated by ultrafiltration. Protein was estimated to be ~0.1 µg; (*d*) component B was isolated from 72 liters of bacterial culture. After the second cycle of affinity chromatography, the Lac-eluted fractions were concentrated to dryness on ultrafiltration membrane PM10. The sample was extracted with 150 µl of sample buffer without β-mercaptoethanol. The sample (45 µl) was applied to lane *d* under nonreducing conditions. (*e*) The same sample as in lane *d* was treated with 10 mM β-mercaptoethanol. (*f*) Autoradiography of ^{125}I-labeled BJ38 (3,000 cpm) described in Materials and Methods. XAR-5 film was exposed for 3 d and then developed. Lanes *a–e* were analyzed by silver stain.

staining of purified BJ38 (Fig. 2, lane *d*) with the silver reagent was compared with the intensities of staining with known amounts of carbonic anhydrase (M_r ~31,000). On this basis, it was estimated that we could isolate 1 µg of BJ38 from 24 liters of *B. japonicum* culture.

To characterize the properties of BJ38, the protein was radiolabeled with ^{125}I. The reaction was carried out in the presence of 0.1 M Lac, which may protect the carbohydrate binding activity during the iodination. After removal of unincorporated ^{125}I and Lac, the radioactive polypeptide bound to Lac-Sepharose and was eluted with 0.1 M Lac (Fig. 1, inset). SDS-PAGE analysis yielded a single band (M_r ~38,000)
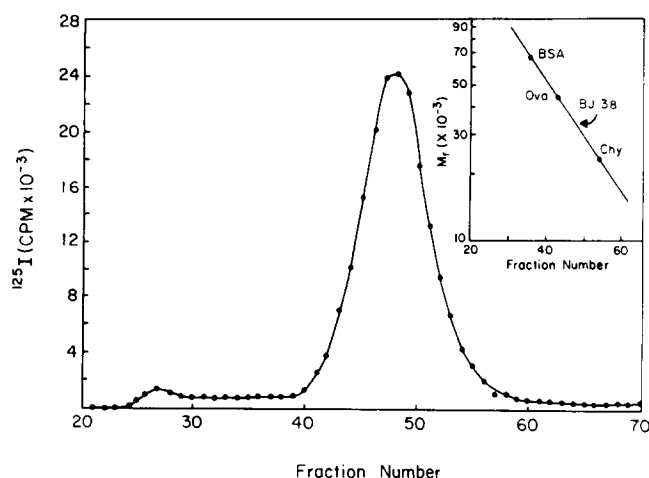

Figure 3. Gel filtration of ^{125}I-labeled BJ38 on Sephadex G-100 column. The column (1.5 × 114 cm) was developed with PBS containing 0.1 M Lac. Fractions of 2 ml were collected. (*Inset*) Semilogarithmic plot of the molecular masses versus elution volumes of calibration standards, blue dextran, bovine serum albumin (*BSA*, M_r 66,000), ovalbumin (*Ova*, M_r 43,000), and chymotrypsinogen (*Chy*, M_r 23,000).

*Table I. Effect of Saccharides on BJ38 Binding to Lac-Sepharose Beads**

Saccharide	Bound	Binding
	cpm	*%*
No hapten	6,420 ± 132	100
Glucose	6,005 ± 7	93 ± 0
Mannose	6,420 ± 169	99 ± 2
Galactose	1,270 ± 98	19 ± 1
Galactosamine	3,895 ± 261	60 ± 4
N-Acetyl-D-galactosamine	4,970 ± 56	77 ± 1
Lactose	825 ± 120	12 ± 1
Melibiose	2,540 ± 155	39 ± 2
Sucrose	5,745 ± 106	89 ± 1

* Aliquots of 0.2 ml of Lac-Sepharose bead suspension (1:1 [vol/vol]) were incubated with 0.1 ml of various saccharides or PBS (as control) and 0.1 ml of ^{125}I-labeled BJ38 (1.5 × 10^4 cpm) for 4 h at room temperature. Samples were washed three times with PBS. The amount of radioactivity associated with the beads was determined. All saccharides were added to a final concentration of 50 mM. The data represent the means of triplicate determinations ± SD.

upon autoradiography (Fig. 2, lane *f*). Thus, ^{125}I-labeled BJ38 exhibited the same molecular and binding properties observed for the unlabeled protein. Routinely, a specific activity of ~1 × 10^8 cpm/µg was obtained for ^{125}I-labeled preparations of BJ38.

When ^{125}I-labeled BJ38 was subjected to gel filtration on Sephadex G-100 equilibrated with PBS containing 0.1 M Lac, a single component was observed (Fig. 3). A semilogarithmic plot of the molecular masses of standard proteins and their elution volumes on the same column showed that the position of elution of ^{125}I-labeled BJ38 corresponded to that of a polypeptide M_r ~38,000 (Fig. 3, inset). Similar results were obtained both in the presence and in the absence of Lac. These data indicate that in nondenaturing solvents, the BJ38 molecule exists in the monomeric form of the M_r ~38,000 polypeptide.

Saccharide-binding Specificity of BJ38

To probe the sugar-binding specificity of BJ38, the ^{125}I-labeled protein was passed over a Lac-Sepharose column. Various saccharides were tested for their capacity to elute the bound radioactive polypeptide. When the column was developed sequentially with 50 mM of glucose, mannose, N-ace-
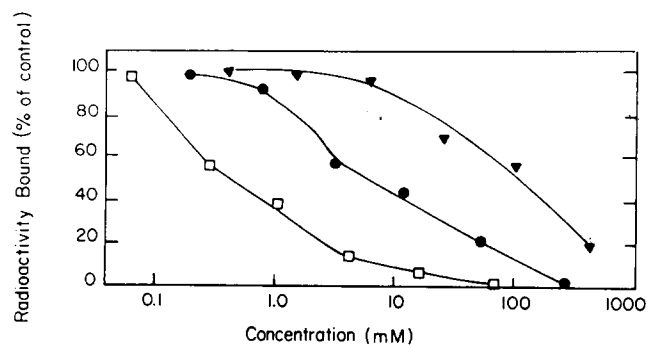

Figure 4. Dose-response curves of the effects of various saccharides on the binding of ^{125}I-labeled BJ38 to Lac-Sepharose beads. (▼) N-Acetyl-D-galactosamine; (●) galactose; (□) lactose.

Table II. Effect of Chelating Agents on BJ38 Binding to Lac-Sepharose Beads*

Chelator	Bound	Binding
	cpm	*%*
No chelator	6,925	100
10 mM EDTA	5,835 ± 77	84 ± 1
10 mM EDTA + 50 mM Lac	1,276 ± 468	18 ± 7
1 mM EDTA	5,880 ± 593	99 ± 8
1 mM EDTA + 50 mM Lac	1,087 ± 120	15 ± 1
10 mM EGTA	5,720 ± 70	82 ± 1
10 mM EGTA + 50 mM Lac	993 ± 93	14 ± 1
1 mM EGTA	6,560 ± 141	94 ± 2
1 mM EGTA + 50 mM Lac	1,144 ± 147	16 ± 2

* Details for the Lac-Sepharose bead binding assay were as described in Table I, replacing saccharides for chelating agents.

tyl-D-galactosamine, and galactose, a prominent radioactive peak was observed only upon the addition of Gal. These results indicate that the binding specificity is restricted to Gal- and Lac-containing glycoconjugates.

Alternatively, the binding specificity of BJ38 could be more quantitatively assessed by comparing the capacity of various saccharides to inhibit the binding of the ^{125}I-labeled protein to Lac-Sepharose beads. Similar to radioimmunoassays, the assay conditions for these studies were chosen such that $\sim$40–50% of the total radioactivity added was bound by the Lac-Sepharose beads in the absence of any hapten inhibitor. At a concentration of 50 mM, there was strong inhibition of BJ38 binding to Lac-Sepharose beads by Gal and Lac (Table I). In contrast to Lac (Gal-β1,4-Glc) (12% of control binding), melibiose (Gal-α1,6-Glc) showed less inhibition (40% of control binding). Derivatives of Gal at C-2, galactosamine, and N-acetyl-D-galactosamine, showed much less inhibition (60–80% of control binding). Finally, sucrose, mannose, and glucose showed essentially no inhibition (Table I).

More refined dose-response curves were carried out for a few inhibitory saccharides (Fig. 4). Lactose was the most potent hapten inhibitor, with half-maximal inhibition at 0.46 mM. While Lac and Gal were both inhibitory, the former was $\sim$13-fold stronger in terms of relative affinities of binding to BJ38. More strikingly, there was an even greater difference in the binding of BJ38 to Gal versus GalNAc; half-maximal inhibition was achieved at 6 mM for Gal, whereas the corresponding value was 110 mM for GalNAc (Fig. 4). All of these results confirm the conclusions obtained using different saccharides to elute ^{125}I-labeled BJ38 from Lac-Sepharose and emphasize the details of specificity with respect to the equatorial group at the C-2 position of Gal.

Effect of pH and Chelating Agents on Saccharide Binding of BJ38

The effect of pH on the binding of BJ38 was tested using Lac-Sepharose beads and the ^{125}I-labeled protein. The pH profile exhibited a broad optimum between pH 5 and 8 (data not shown). Above and below these pHs, there was a gradual decrease in the amount of ^{125}I-labeled BJ38 bound to the Lac-Sepharose beads.

The question arose whether BJ38 had an intrinsic requirement for divalent cations such as Ca^{2+} ion in order to bind

the saccharide. To test this, the binding of ^{125}I-labeled BJ38 to Lac-Sepharose beads was determined in the presence and absence of EGTA or EDTA. Neither chelator affected the binding of ^{125}I-labeled BJ38 to any appreciable extent (Table II), nor did the presence of EDTA or EGTA alter the ability of Lac to inhibit the binding of ^{125}I-labeled BJ38 to the beads. These results indicate that BJ38 most probably does not require divalent cations for binding.

Loss of BJ38 Lectin Activity in Mutants N4 and N6

We have isolated two mutants, designated N4 and N6, from *B. japonicum* on the basis of defective binding to SB-1 cells (7). Since these mutants appeared to have lost or impaired binding in all four of the saccharide-specific assays, it was of obvious interest to test for BJ38 in the N4 and N6 cells. When extracts derived from wild-type R110d cells were fractionated on Lac-Sepharose affinity columns, the bound material could be eluted with Lac and then labeled with ^{125}I. Upon rechromatography on an identical Lac-Sepharose column, the ^{125}I-labeled BJ38 could be identified (Fig. 1, inset, and Fig. 2, lane *f*). In contrast, when extracts from N4 and N6 cells were fractionated and radiolabeled in parallel, we could detect no ^{125}I radioactivity bound to the second Lac-Sepharose affinity column and eluted by the application of Lac (Fig. 5). It appears, therefore, that the mutants N4 and N6 have lost the BJ38 lectin activity.

Discussion

The results obtained in the present study indicate that we have purified, from *Bradyrhizobium japonicum*, a carbohydrate binding protein with the following key physical properties. First, the molecular mass of the polypeptide chain, determined by SDS-PAGE under both reducing and nonreducing conditions, was M_r $\sim$38,000. In nondenaturing solvents, the protein chromatographed on gel filtration columns with an apparent molecular mass of $\sim$38,000; therefore, it appears that BJ38 did not form oligomers of the polypeptide subunit. This conclusion should be qualified, however, by the knowledge that the gel filtration experiments were carried out using minute amounts of radiolabeled protein and, thus, the concentration of BJ38 used in our column experi-
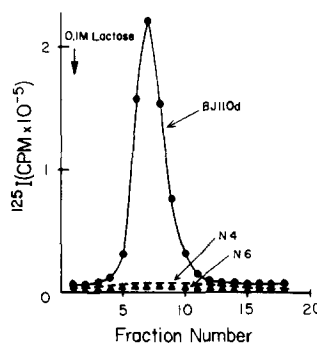

Figure 5. Isolation of BJ38 from the wild-type R110d and comparison with mutants N4 and N6. BJ38 was isolated from 12 liters of bacterial culture as starting material. Bacterial extract was subjected to affinity chromatography on Lac-Sepharose column. The lactose-eluted fractions were concentrated by ultrafiltration to a volume of 0.2 ml. An aliquot of 25 μl was radioiodinated and reapplied to the Lac-Sepharose column. The radioactivity eluted from the column after the addition of lactose was used for BJ38 quantitation. (●) *B. japonicum* R110d sample; (--- and ▲) samples from N4 and N6, respectively.

ments may be below the threshold required for self-association.

Second, two-dimensional gel electrophoresis resolved BJ38 into two different spots, with pI values of 6.4 and 6.8. The relationship between these two polypeptides, of about the same molecular mass but of different isoelectric points, has not been determined. They could represent distinct gene products; alternatively, they may represent posttranslationally modified variants of the same gene product. In any case, the fact that both polypeptides were jointly isolated by Lac-Sepharose affinity chromatography suggests that each polypeptide can bind to carbohydrate. As emphasized above, BJ38 chromatographed to a position corresponding to a polypeptide of M_r ~38,000 in gel filtration experiments, both in the presence and absence of Lac. The result suggests that the two polypeptides do not form oligomers. It argues against the possibility that only one of the polypeptides binds to carbohydrate, and that the other polypeptide is noncovalently associated with the carbohydrate-binding polypeptide.

Third, BJ38 bound specifically to Gal and Gal-containing glycoconjugates. Epimeric sugars such as mannose and glucose did not show any binding. Moreover, alteration of Gal at the C-2 position resulted in drastic lowering of the binding affinity, relative to Gal. For example, GalNAc exhibited 18-fold lower binding than Gal. The binding of the disaccharide Lac, on the other hand, was 13-fold higher than that of Gal. Thus, the binding of GalNAc was some 200-fold weaker than Lac. Consistent with this large difference in affinities, GalNAc failed to release ^{125}I-labeled BJ38 bound to Lac-Sepharose. This capability of BJ38 to distinguish Gal versus GalNAc is similar to that observed in the plant lectin ricin, which binds to Gal with higher affinity than to GalNAc (17). In contrast, soybean agglutinin (20) and discoidin from *Dictyostelium discoideum* (2) both have higher affinities for GalNAc than for Gal, although both saccharides are bound.

The ability of BJ38 to distinguish Gal versus GalNAc is also of particular interest in light of observations documented in the accompanying paper (7). We have found that *B. japonicum* exhibit four saccharide-specific binding activities: (*a*) adsorption to Lac-Sepharose; (*b*) autoagglutination; (*c*) binding to cultured SB-1 cells; and (*d*) adhesion to soybean roots. In all four of these assays, Gal inhibited the binding, while GalNAc did not. Similarly, Lac was a strong inhibitor, whereas melibiose showed much less inhibition (7), consistent with the data obtained for BJ38. At the correlative level, therefore, one likely candidate to account for the carbohydrate binding activities of *B. japonicum* is BJ38. Consistent with this hypothesis, we have found that the mutants N4 and N6, which have lost the carbohydrate binding properties of wild type *B. japonicum*, also appeared to have lost the lectin activity corresponding to BJ38.

Finally, preliminary evidence indicates that BJ38 did not carry enzymatic activities such as β-galactosidase. We have also failed to find identities between the polypeptide molecular mass, pI value, and pH optimum for BJ38 with the corresponding molecular parameters for a number of bacterial galactosidases (23, 24), arabinogalactanases (25), pectin lyase (21), and pectin esterases (4) reported in the literature.

Many hemagglutinins of bacterial origin have been previously identified and characterized (1, 3, 8, 9, 12, 15, 16, 19, 22). These exhibit distinct sugar specificities. Moreover, some bacterial cells often express three or four hemagglutinins, each with a different carbohydrate binding specificity.

A comparison of the data accumulated for BJ38 in the present study with the known properties of other bacterial lectins suggest that we have identified and isolated a new carbohydrate binding protein, BJ38.

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