Human Splenic Galaptin: Physicochemical Characterization†

Ashu Sharma, Richard Chemelli, and Howard J. Allen*
Department of Surgical Oncology, Roswell Park Memorial Institute, Buffalo, New York 14263
Received November 27, 1989; Revised Manuscript Received February 5, 1990

ABSTRACT: Mammalian spleens were previously reported to contain β-galactoside-binding lectins [Allen, H. J., Cywinski, M., Palmberg, R., & DiCioccio, R. (1987) *Arch. Biochem. Biophys. 256*, 523–533]. The aim of the present investigation was to determine the relationship of human splenic galaptin to other β-galactoside-binding lectins identified in other human and animal tissues. Galaptin of subunit molecular mass 14.5 kDa was the only lectin of this type found in human spleen as assessed by SDS–PAGE, RP-HPLC, and Western blot analyses. Three polypeptides of p*I* 4.60, 4.80, and 4.85 were detected by isoelectric focusing of purified galaptin, with the major band having p*I* 4.85. UV spectral analysis indicated the absence of prosthetic groups and gave $A^{1\%}_{1\,cm,\,280} = 5.5$. Circular dichroic analysis suggested the presence of 40% β structure, considerable random coil, and 10% α helix structure. The amino acid composition was very similar to that for human placental galaptin. Amino acid sequence analyses were carried out on V8 protease, CNBr, and iodosobenzoic acid digestion fragments. A total of 94 residues were identified. All sequences determined could be aligned with placental galaptin sequences. We conclude that human splenic galaptin is identical with human placental galaptin. A related polypeptide of molecular mass ~14.5 kDa was found to be present in several different mammalian spleens as assessed by Western blot analysis using a monospecific polyclonal anti-human splenic galaptin antiserum.

In recent years, lectins have been detected, isolated, and characterized for a variety of animal cells and tissues (Barondes et al., 1988; Monsigny et al., 1988). Several of these lectins fall into a group characterized as thiol-dependent, cation-independent, developmentally regulated lactose-inhibitable lectins (Barondes, 1984) and have been termed galaptins (Harrison & Chesterton, 1980). A major galaptin species is frequently isolated as a native 30-kDa dimer (Allen et al., 1987a), although occasionally the 14-kDa monomeric form is obtained (Hirabayashi & Kasai, 1984). Other molecular mass species have also been reported (Cerra et al., 1985; Sparrow et al., 1987).

Immunologic studies showed that many of these soluble β-galactoside-binding lectins present in different species and tissues are related (Hirabayashi et al., 1987a; Allen et al., 1987a). The homologous nature of the galaptins has been confirmed by amino acid and nucleotide sequencing studies. Direct amino acid sequence analyses have been reported for galaptin isolated from electric eel and human placenta (Paroutaud et al., 1987), bovine heart (Southan et al., 1987), chick embryo (Hirabayashi et al., 1987b), human placenta (Hirabayashi & Kasai, 1988), and human lung (Gitt & Barondes, 1986). These amino acid sequence determinations confirmed that galaptins belong to a homologous family of gene products. Several cDNA clones and their nucleotide sequences have been reported for galaptin from different sources such as human hepatoma (Gitt & Barondes, 1986; Abbott & Feizi, 1989), human promyeloleukemia and placenta (Couraud et al., 1989), human lung (Hirabayashi et al., 1989), chick (Ohyama & Kasai, 1988) bovine fibroblasts (Abbott et al., 1989), rat lung and uterus (Clerch et al., 1988), and murine fibroblasts (Wilson et al., 1989). The results of these studies confirm that a high degree of homology exists between galaptins present in different species. In most cases, the data support the conclusion that a single galaptin gene is present in different species. However, other observations on soluble β-galactoside-binding lectins in animal tissues reveal complexities yet to be clarified, such as the presence of multiple lectins in rat (Cerra et al., 1985) and human lung (Sparrow et al., 1987), the reported immuno-cross-reactivity of anti-bovine galaptin monoclonal antibody (Carding et al., 1985; Abbott et al., 1989), the bifunctional nature of the murine CBP35-IgE receptor (Laing et al., 1989; Raz et al., 1989), and the bifunctional nature of the elastin receptor (Mecham et al., 1989).

We have previously reported the presence of galaptins in mammalian spleens (Allen et al., 1987b) and in human peripheral leucocytes and HL-60 cells (Allen et al., 1986). Whereas a 30-kDa dimeric galaptin was isolated from human spleen, a monomeric 14.5-kDa galaptin was synthesized in vitro by human peripheral leucocytes. Promyelocyte HL-60 cells, however, synthesized two β-galactoside-binding proteins of native molecular mass 28 and 19.5 kDa. The 28-kDa galaptin was composed of subunits separable from the 19.5-kDa polypeptide. As part of our studies to clarify the relationship between these lectins, we report here the physicochemical characterization of human splenic galaptin.

Materials and Methods

Isolation and Purity of Galaptin. Human spleen was obtained at splenectomy and stored at −20 °C until utilized. Frozen spleen was cut into small fragments with the aid of a hacksaw and homogenized in 10 volumes of ice-cold acetone–0.01 M ME.[1] The homogenate was filtered on a Büchner funnel and the residue stored at −20 °C until utilized. Galaptin was isolated from the acetone powder by affinity chromatography on asialofetuin–Sepharose essentially as

† Supported by NIH Grant CA42584 to H.J.A. and by NIH Institutional BRSG Grant 2S07RR05648-22.
* Author to whom correspondence should be addressed.

[1] Abbreviations: ME, mercaptoethanol; TBS, 10 mM Tris–25 mM NaCl, pH 7.5; DTT, dithiothreitol; IEF, isoelectric focusing; RP-HPLC, reverse-phase high-pressure liquid chromatography; RT, room temperature; PBS, 50 mM PO_4–100 mM NaCl; RCM, reduced and carboxymethylated; DW, deionized water; PVDF, poly(vinyl difluoride); IBA, iodosobenzoic acid; TFA, trifluoroacetic acid.

0006-2960/90/0429-5309$02.50/0

previously described (Allen et al., 1987b) with the following modifications: the affinity column load was not made 0.5 M in NaCl; prior to elution with lactose, the affinity column was washed with TBS–0.01 M ME; galaptin was eluted with TBS–0.01 M ME–0.1 M lactose. The lactose-eluted galaptin was loaded onto DEAE-Sephacel (0.5–1-mL bed) equilibrated with TBS–0.01 M ME, and the column was washed with TBS–0.01 M ME. The column was then washed with TBS–0.01 M ME–0.1 M lactose–50% glycerol and stored at −20 °C until utilization of galaptin. On occasion, lactose coupled to Sepharose via divinyl sulfone (Allen & Johnson, 1977) was used as an effective affinity adsorbent.

Galaptin was recovered from DEAE-Sephacel by washing the column with TBS–0.01 M ME followed by elution with TBS–0.01 M ME–0.2 M NaCl. In some cases, 0.001 M DTT replaced ME.

Purity of galaptin was assessed by SDS–PAGE, IEF, and RP-HPLC. Samples were prepared for SDS-PAGE by reduction in 0.6 M Tris–0.03 M DTT–0.037 M EDTA, pH 8.6, followed by alkylation with 0.06 M iodoacetamide, all under N_2 at RT. Following exchange to sample buffer, SDS–PAGE was carried out on 8–25% acrylamide Phastgels according to Pharmacia protocols. Protein bands were visualized with Coomassie blue or silver staining. Calibration proteins were run in parallel for construction of a standard curve.

IEF was carried out on pH 3–10 and pH 4–6.5 gels using the Pharmacia Phast system. IEF and Coomassie blue and silver staining were done according to the manufacturer's protocol. Calibration proteins were run in parallel for construction of a standard curve.

RP-HPLC was carried out on a 2.1 × 30 mm Aquapore RP-300 (C-8) column. Solvent A was 0.1% TFA in DW; solvent B was 0.082% TFA–70% acetonitrile in DW. A 5-μg quantity of galaptin or RCM-galaptin was dissolved in 30 μL of solvent A for loading. Gradient elution was carried out, 0–100% solvent B in 70 min, at 200 μL/min. The effluent was monitored at 214 nm.

Antiserum and Western Blot Analysis. Rabbit antiserum was raised against the 14.5-kDa galaptin subunit prepared from human spleen. Affinity-purified galaptin was eluted from DEAE-Sephacel and subjected to preparative slab gel SDS–PAGE as described for analytical gels (Allen et al., 1987b). The region of the gel containing the 14.5-kDa subunit was determined by staining a portion of the gel with Coomassie blue. This region was extracted by homogenization in H_2O with a Brinkmann Polytron homogenizer. After a protein assay, the soluble fraction was lyophilized. The rabbit was injected with 350 μg of protein in Freund's complete adjuvant followed by booster injections of 140 μg of protein in Freund's incomplete adjuvant.

Western blot analyses were carried out for extracts of spleen obtained from different animals. Spleens were obtained from animals undergoing necropsy or from local slaughterhouses. Tissues were homogenized in 1.2 M Tris–0.037 M EDTA–2% SDS–10 M urea–0.001 M DTT–0.25 mM phenylmethanesulfonyl fluoride, pH 8.6. After centrifugation at 18000*g*, 20 min, 20 °C, solid DTT was added to the soluble extract to give a final concentration of 0.03 M, and the mixture was incubated at RT, under N_2, for 2 h. Solid iodoacetamide was then added to give a concentration of 0.1 M, and the mixture was incubated at RT, under N_2, for 30 min. The extracts were equilibrated with sample buffer by passing them through desalting gel filtration columns prior to SDS–PAGE on 12% acrylamide slab gels (Laemmli, 1970). The gel was blotted onto nitrocellulose by using a Hoefer, Inc., semidry blotting apparatus following the manufacturer's directions. The nitrocellulose was blocked with 5% nonfat dry milk. Immunodetection of blotted protein was carried out with anti-galaptin serum (1:1000) followed by goat anti-rabbit IgG–peroxidase conjugate (1:500) with 4-chloro-1-naphthol as peroxidase substrate.

UV and CD Spectra. UV spectra were determined on a Perkin-Elmer Lambda 4A spectrophotometer at RT. Salt-free lyophilized samples were dissolved in PBS, pH 7.3, at 200 μg/mL. CD spectra were obtained on a Jasco Model 500A at RT. Salt-free lyophilized samples were dissolved at 200 μg/mL in 0.05 M PO_4, pH 7.2, and scanned from 260 to 200 nm. Some samples included 0.001 M DTT and/or 0.01 M lactose. The spectra were compared to those for poly(L-lysine) to estimate the percentage of α helix, β structure, and random coil structure (Greenfield & Fosman, 1969).

Amino Acid Sequence Analysis. (A) Reduction and Carboxymethylation. Galaptin was dissolved in 0.55 M Tris–10 M urea–0.037 M EDTA, pH 8.5, to give 2 mg/mL. Mercaptoethanol, 8 μL/mg of galatpin, was added and the sample was incubated under N_2 for 4 h at 37 °C. Then 4 mg of iodoacetic acid/mg of galaptin was added, and the sample was incubated in the dark under N_2 for 30 min at 37 °C. Desired buffer exchanges were then carried out by using Centricon 10 centrifugation devices, and the RCM-galaptin was stored at −20 °C until utilized.

(B) V8 Protease Digestion and Separation of Peptide Fragments. Native or RCM-galaptin was equilibrated with freshly prepared 0.025 M $(NH_4)_2CO_3$–0.1% SDS, pH 7.8. Typically for digestion, 10 μg of V8 protease was added to 100 μg of galaptin in a 100-μL volume. Digestion was allowed to proceed overnight at RT. Controls included V8 protease lacking galaptin and galaptin lacking V8 protease. After digestion, the samples were mixed with an equal volume of electrophoresis sample buffer (0.05 M Tris–0.1% SDS–5% ME–20% glycerol–0.2% bromphenol blue, pH 6.8) and boiled for 3 min.

Peptide fragments resulting from V8 protease digestion were prepared for sequencing by electrophoresis and electroblotting based upon the method of Matsudaira (1987). SDS–PAGE was performed according to Laemmli (1970) in 14 cm × 9 cm × 1.5 mm 15% acrylamide gels. The upper chamber running buffer contained 0.002% thioglycolic acid, and the gel was preelectrophoresed for 20 min at 250 V prior to sample loading. Typically 10–15 μg of digested galaptin was loaded onto each lane. Control digests and molecular weight markers were also run. Electrophoresis was carried out at 250 V until the dye reached within 1 cm of the gel bottom. After being destained and washed with DW, the PVDF blots were air-dried. Peptide bands of interest were cut out with a razor blade and stored desiccated at −20 °C.

(C) CNBr Cleavage. RCM-galaptin, 15 μg/lane, was electrophoresed, electroblotted, and stained as described for V8 protease fragments. CNBr cleavage was based upon the method of Scott et al. (1988). The protein band was cut out and transferred to a 1.5-mL microfuge tube (protein band up) containing 60–70 μL of 1.6% CNBr in 70% formic acid (sufficient volume to cover the membrane). The tube was sealed under N_2 and incubated at RT, overnight in the dark. The sample was then dried in vacuo, washed twice with DW, dried in vacuo, and stored desiccated at −20 °C.

(D) IBA Cleavage. RCM-galaptin, 15 μg/lane, was electrophoresed, electroblotted, and stained as described for V8 protease fragments. Iodosobenzoate cleavage was based upon the reagent methodology of Mahony et al. (1981). The protein

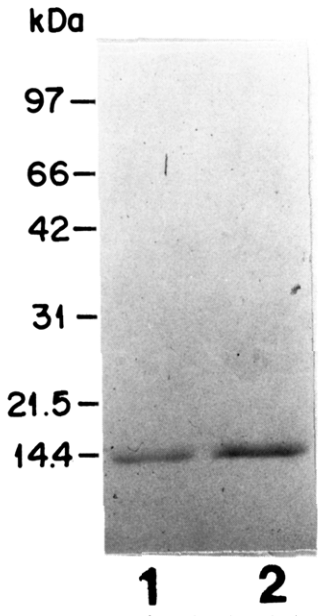

FIGURE 1: SDS–PAGE of splenic galaptin. Galaptin was loaded on an 8–25% polyacrylamide Phastgel run in the Pharmacia, Inc., Phastsystem. The gel was stained with Coomassie blue. Lane 1, 75 ng of galaptin; lane 2, 150 ng of galaptin. Calibration markers are indicated.

band was cut out of the blot and placed on a Teflon support, protein band up, elevated 1 cm above the bottom of a petri dish. The band was covered with IBA reagent (6 mg of IBA in 1 mL of 4 M guanidine hydrochloride prepared in 80% acetic acid) with excess reagent placed in the petri dish. The dish was covered and incubated at RT, overnight in the dark. The bands were then prepared for storage as above.

Sequencing Procedures. Sequencing was carried out on an Applied Biosystems 470A gas-phase sequencer with an on-line 120A HPLC analyzer for PTH amino acid derivatives. Trifluoroacetic acid treated glass fiber filters were coated with 3 mg of Polybrene and then precycled in the sequencer. The excised PVDF blots were prewashed with ethyl acetate and then placed on the precycled glass filters with the peptide side up. The peptides were sequenced by using the standard O3RPTH program modified for a 100-μL sample loop.

Other Procedures. Amino acid analyses were carried out with a Waters workstation and a Waters Pico-Tag system in the RPMI Biopolymer Facility according to the manufacturer's protocol for PTH amino acid derivatives. Samples were hydrolyzed for 24 h at 115 °C in 6 N HCl–phenol vapors.

Hemagglutination assays were carried out as before (Allen et al., 1987b). Protein was assayed by using a Coomassie blue reagent with ovalbumin as a standard.

Materials. Sepharose and DEAE-Sephacel were obtained from Pharmacia (Piscataway, NJ). An Aquapore RP-300 column was obtained from Applied Biosystems (Foster City, CA). Goat anti-rabbit IgG–peroxidase conjugate was from Kirkegaard & Perry (Gaithersburg, MD). Centricon 10 devices were obtained from Amicon (Danvers, MA). V8 protease was obtained from Calbiochem (La Jolla, CA). PVDF (Immobilon) membranes were obtained from Millipore (Bedford, MA). Coomassie blue reagent was obtained from Bio-Rad (Richmond, CA). All other reagents were the highest grade available.

RESULTS

Isolation and Purity of Galaptin. Galaptin has been isolated from human spleens of varying pathological states with yields of 20–130 mg/kg of wet spleen. Isolated galaptin, regardless

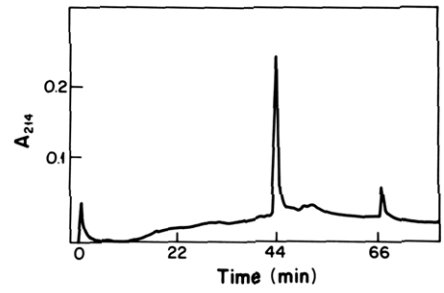

FIGURE 2: RP-HPLC of splenic galaptin. RCM-galaptin, 5 μg, was loaded on an Aquapore RP-300 column and eluted as described under Materials and Methods.

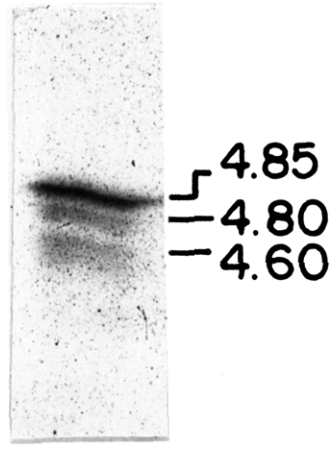

FIGURE 3: IEF of splenic galaptin. Isoelectric focusing was carried out on a pH 4–6.5 IEF Phastgel as described under Materials and Methods. The gel was silver-stained. The p*I* of the observed polypeptides is indicated.

of spleen histopathology, had a minimum hemagglutinating activity of 0.1–1 μg/mL when assayed with trypsinized glutaraldehyde-fixed rabbit erythrocytes. Hemagglutination was inhibited by lactose. SDS–PAGE showed the presence of a single polypeptide of mass 14.5 kDa (Figure 1). A single polypeptide that eluted at 44 min was detected by RP-HPLC (Figure 2). IEF revealed the presence of three distinct bands corresponding to p*I* 4.60, 4.80, and 4.85 (Figure 3). Storage of native galaptin at 4 °C resulted in increased staining intensity of the more acidic bands (not shown). Western blot analysis using the antiserum described here revealed the presence of a single immunoreactive polypeptide of mass 14.5 kDa in human spleen extracts (Figure 4). A similar polypeptide was immunodetected in all mammalian spleens analyzed (baboon, calf, pig, lamb, cat, bat, rat, mouse, guinea pig). Rabbit spleen extracts showed very weak reactivity. Trout spleen, free-living *Amoeba*, and land slug extracts did not react with the antiserum.

Spectral Analysis. The UV spectrum of galaptin was typical of proteins lacking prosthetic groups. The absorption coefficient was found to be $A^{1\%}_{1\text{ cm},280} = 5.5$. The absorption coefficient for galaptin in the Coomassie blue dye binding assay was found to be $A^{1\%}_{1\text{ cm},595} = 7.0$. Analysis of the CD spectra suggests that the native protein contains 40% β sheet structure and 10% or less α helix, with the remainder random coil. Similar spectra were obtained in the presence and absence of DTT and 10 mM lactose.

Amino Acid Composition and Sequence of Galaptin. The amino acid composition of galaptin is given in Table I. The minimum subunit molecular mass, assuming an average amino acid molecular mass of 105 Da, is 13 860 Da. The amino acid composition is very similar to that reported for human placental lectin (Hirabayashi et al., 1987c).

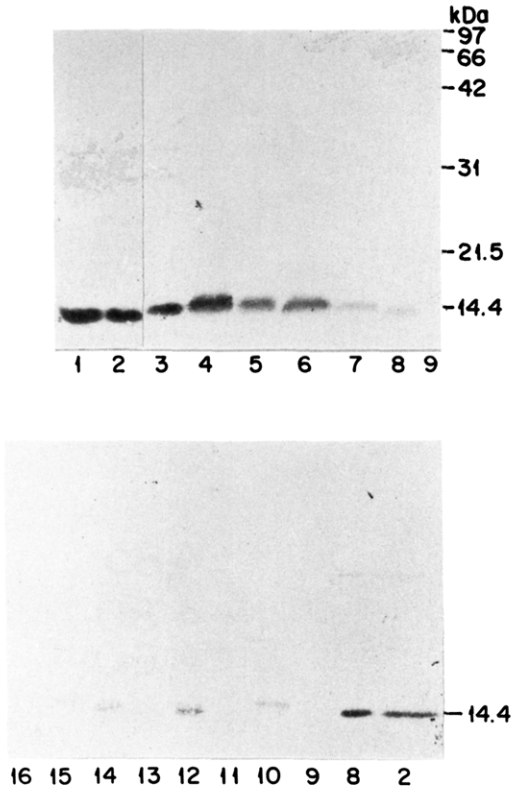

FIGURE 4: Western blot analysis of tissue extracts. The blots were probed with anti-splenic galaptin as described under Materials and Methods. Calibration markers are indicated. Lane 1, 20 ng of human splenic galaptin; 2, 10 ng of galaptin; 3, human spleen extract; 4, baboon; 5, cat; 6, calf; 7, lamb; 8, pig; 9, rabbit; 10, rat; 11, whole slug extract; 12, mouse; 13, trout; 14, guinea pig; 15, bat; 16, *Amoeba* extract.

Table I: Amino Acid Analysis of Splenic Galaptin[a]

residue	determined	lit. value[b]	residue	determined	lit. value[b]
Asx	22	21	Tyr	2	2
Glx	11	10	Val	9	9
Ser	5	5	Met	1	1
Gly	12	12	Cys[c]	3	5
His	2	2	Ile	3	4
Arg	5	5	Leu	12	11
Thr	4	3	Phe	10	9
Ala	16	13	Lys	7	7
Pro	8	6	Trp[d]		1

[a] Reported as residues per 132 amino acid residues. [b] Literature values reported by Hirabayashi et al. (1987c). [c] Estimated value. [d] Not determined.

Attempts to directly sequence intact galaptin present in solution and on PVDF blots failed to generate a signal through the first three cycles of analysis, indicating the presence of a blocked NH_2 terminus. Subsequent strategy was based on digestion of galaptin with V8 protease, separation of peptide fragments by electrophoresis, electroblotting onto PVDF, detection of peptides by Coomassie blue staining, and direct sequencing of the stained oligopeptides. Despite the presence of a high proportion of Glx and Asx residues, galaptin was quite resistant to V8 protease digestion. Digestion never went to completion, and the resulting fragments yielded a fairly simple profile upon staining of PVDF blots. A representative separation is shown in Figure 5. Direct sequencing following CNBr degradation was based on the expectation that a single free NH_2 terminus would be produced as a result of cleavage at the single methionine residue. Direct sequencing following IBA cleavage was based on the probability that a single tryptophan residue might be present (Hirabayashi & Kasai,

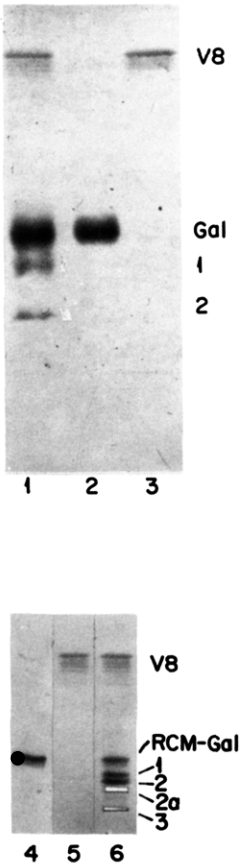

FIGURE 5: PVDF blots of V8 protease digestion fragments of galaptin. Lane 1, fragments from native galaptin digestion; lane 2, native galaptin; lane 3, fragments from V8 protease autodigestion; lane 4, RCM-galaptin; lane 5, V8 protease autodigestion; lane 6, fragments from RCM-galaptin digestion (lower bands excised for sequencing). The peptide bands are numbered as given in Table II. Residual galaptin and RCM-galaptin, galaptin 1, and RCM-galaptin 1 and 2 were found to have blocked NH_2 termini.

Table II: Spleen Galaptin Sequenced Peptides

peptide[a]	source	no. of residues identified
Glu-3	RCM-Gal-V8-2A[b]	15
Glu-3	RCM-Gal-V8-2A	26
Glu-5	Gal-V8-2	20
Glu-5	RCM-Gal-V8-3	20
Glu-5	RCM-Gal-V8-2A	15
Glu-5	RCM-Gal-V8-2A	15
Glu-6	Gal-V8-2	14
Glu-6	Gal-V8-2	20
Glu-6	RCM-Gal-V8-3	20
CNBr-2	RCM-Gal-CNBr	14
IBA-2	RCM-Gal-iodoso-2	19
IBA-3	RCM-Gal-iodoso-2	19

[a] Peptide sequences are given in Figure 6. [b] PVDF blot band 2A derived from V8 protease digestion of RCM-galaptin.

1988). Therefore, one sequence of primary signals might be obtained with weaker signals present due to inefficient cleavage at tyrosine residues.

The procedures yielded six sequenceable peptides with several overlaps, which resulted in the identification of 94 residues. The nomenclature for these peptides, their sources, and the number of residues identified are summarized in Table II. In some cases, a given peptide was sequenced up to four different times. Amino-terminal peptide fragments generated by V8 protease digestion were identifiable in that they yielded no signal upon attempted sequencing. Some bands obtained from V8 protease digestion contained two peptide fragments; e.g., Glu-3 and Glu-5 were both present in PVDF band

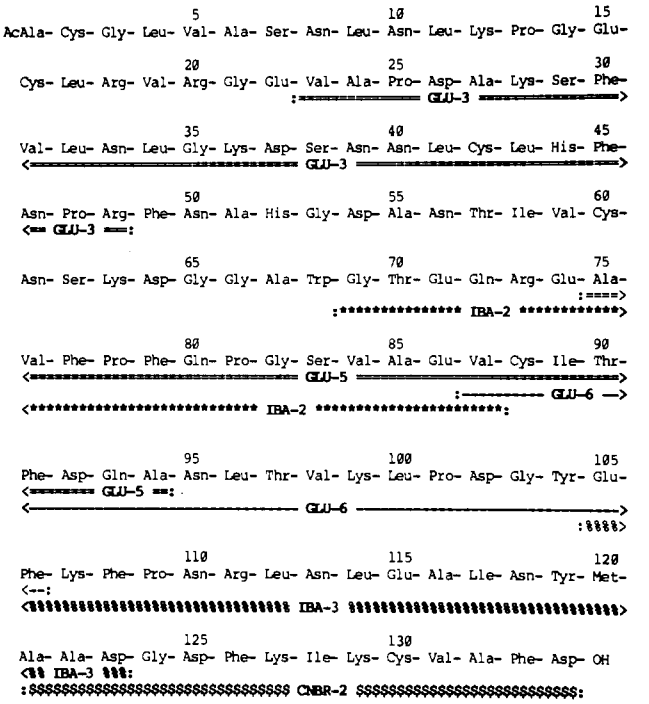

FIGURE 6: Amino acid sequence of splenic galaptin. The complete sequence for human placental galaptin is given with residue numbers indicated (see Results). The sequences for splenic galaptin peptides and their overlaps are shown. Glu-3 and Glu-5, =; Glu-6, —; CNBr-2, $$$; IBA-2, ***; IBA-3, %%%.

RCM-Gal-V8-2A. However, the fragments were present in unequal amounts and generated considerably different signals. Therefore, it was possible to assign unambiguous sequences. The same was true for the products of IBA cleavage. A major and a minor set of signals were generated, resulting from primary cleavage at a tryptophan residue and minor cleavage at a tyrosine residue.

The determined sequences are presented in Figure 6. The identities of the peptides are shown with regions of overlap indicated. In every case, the determined sequences could be aligned with sequences determined for human placental lectin (Hirabayashi & Kasai, 1988; Courad et al., 1989). Therefore, the residue number assigned corresponds to that for placental lectin.

Discussion

The aim of the present investigation was to determine the relationship of human splenic galaptin to other β-galactoside-binding lectins identified in other human and animal tissues. Although multiple lectins were identified in human lung (Sparrow et al., 1987) and two lectins were reported to be present in promyelocytic HL-60 cells (Allen et al., 1986; Couraud et al., 1989), we were able to isolate only one galaptin from human spleen. The native galaptin is a 30-kDa dimer (Allen et al., 1987b) consisting of 14.5-kDa subunits. Purity of splenic galaptin was confirmed by RP-HPLC of native and RCM-galaptin. The antiserum described here reacted with no other polypeptides present in human spleen extracts except the 14.5-kDa polypeptide. The antiserum also reacted with a polypeptide of ~14.5 kDa present in several different mammalian spleen extracts. Hence, the galaptins previously described (Allen et al., 1987b) are structurally related.

The weak reactivity of the rodent spleen extracts is probably due to greater differences in their galaptin relative to the other samples as a result of greater phylogenetic distance. The lack of reactivity for *Amoeba*, slug, and trout may reflect increased phylogenetic distance or lack of galaptin. Occasionally, very weak reactivity was detected with rabbit spleen extracts. Although rabbit tissues are reported to contain galaptin (Catt & Harrison, 1985), apparently our rabbit polyclonal antiserum does not contain significant autoantibodies directed against epitopes present on rabbit galaptin.

We have found that if samples for SDS–PAGE are not adequately alkylated or if thiol-reducing agents are not present in the stacking and separating gels, galaptin-related polypeptides of variable molecular mass may be detected. This is the case for Western blot analysis, fluorographic analysis of immunoprecipitates, and silver and Coomassie blue staining of affinity-purified galaptin. It is possible that the SH groups present in galaptin easily form disulfide bonds and generate homo- and heteroaggregates. This phenomenon may be partly responsible for detection of some of the higher molecular mass species of galaptin that are occasionally reported in the literature.

Although isolated galaptin was homogeneous as evaluated by SDS–PAGE and RP-HPLC, IEF revealed the presence of three protein bands. The abundance of the more acidic bands increased upon storage of galaptin at 4 °C. A similar observation was noted by Clerch et al. (1988) for rat lung galaptin. These data confirm previous observations on splenic galaptin (Allen et al., 1987b). How these various acidic bands appear and change with time is unknown. It is possible that the amide groups of some of the Asn residues are labile and are lost during storage, generating more acidic carboxyl groups.

The results of CD analysis lead to the prediction of a high content of β structure and very little α helix in splenic galaptin. This is in agreement with data for chick galaptin (Hirabayashi et al., 1987b) but differs from the conclusion of Clerch et al. (1988) for rat lung galaptin. In view of the observations that galaptin is frequently found at extracellular sites (Barondes, 1984), it is worth noting that a high β structure content is one of the characteristics of extracellular proteins (Nishikawa et al., 1983). The presence of DTT or lactose did not appear to affect the secondary structure of galaptin.

The subunit mass, p*I*, and amino acid composition of human splenic galaptin were found to be very similar to those of human placental lectin. Therefore, the sequencing strategy described here was based in part on the reported placental lectin sequence (Hirabayashi & Kasai, 1988; Courad et al., 1989). Of significant utility to this strategy was the utilization of SDS–PAGE and electroblotting onto PVDF membranes as an alternative to RP-HPLC for the preparation of peptide fragments. Interestingly, splenic galaptin proved to be quite resistant to protease digestion. Digestion with trypsin, submaxillaris protease, and endoproteinase Arg-C failed to yield peptide fragments in sufficient yield for sequencing, whether analyzed by RP-HPLC or SDS–PAGE. V8 protease digestion did yield large fragments separable by SDS–PAGE and amenable to partial sequencing. Contamination with V8 protease autodigestion fragments was ruled out by including V8 protease controls for autodigestion and SDS–PAGE.

The amino terminus of splenic galaptin was found to be blocked, as has been reported for other galaptins. Since only one methionine residue was present in the 14.5-kDa polypeptide, in situ CNBr digestion yielded one fragment with a free amino terminus that could be completely sequenced. In situ IBA degradation yielded two fragments amenable to simultaneous sequencing. A major peptide (IBA-2) was sequenced, which we assume to be derived from cleavage at a single tryptophan residue. This peptide was found to overlap with the Glu-5 peptide. A minor IBA fragment (IBA-3) was present, which overlapped with the Glu-6 and CNBr-2 pep-

tides. This fragment resulted from cleavage at Tyr-104.

Subunit molecular mass and amino acid composition data suggested that 131–138 residues should be present in the galaptin subunit. Of these, 94 were identified. All sequences determined aligned with sequences reported for the placental lectin.

There is a consensus N-glycosylation site at amino acid residues 95–97. We have not ruled out the possibility of an *N*-glycoside at this site. However, others have failed to find covalently linked carbohydrate in this group of galactoside-binding lectins (Hirabayashi & Kasai, 1988; Courad et al., 1989).

We conclude that human splenic galaptin is identical with the placental galaptin and probably corresponds to the galaptin reported to be present in a wide variety of human cells and tissues (Allen et al., 1987a). Human splenic galaptin belongs to a family of related proteins present in other mammalian spleens. This group of proteins represents another addition to the single-gene-encoded 14.5-kDa family [cf. Abbot and Feizi (1989)] of β-galactoside-binding proteins.

The function of galaptin is unknown but is most certainly related to its carbohydrate-binding specificity. Therefore, efforts are underway to more adequately understand the characteristics of the splenic galaptin binding site (Ahmed et al., 1990; Lee et al., 1990).

ACKNOWLEDGMENTS

We thank the UGI Service and Charlotte Pollard, Pathology Department, for assistance in obtaining spleen specimens. We also thank Dr. J. Bello, Biophysics Department, for assistance with CD analysis.

REFERENCES

Abbott, W. M., & Feizi, T. (1989) *Biochem. J. 259*, 291–294.

Abbott, W. M., Mellor, A., Edwards, Y., & Feizi, T. (1989) *Biochem. J. 259*, 283–290.

Ahmed, H., Allen, H. J., Sharma, A., & Matta, K. L. (1990) *Biochemistry* (following paper in this issue).

Allen, H. J., & Johnson, E. A. Z. (1977) *Carbohydr. Res. 58*, 253–265.

Allen, H. J., Kachurek, D., Bulera, S., Kisailus, E., & DiCioccio, R. (1986) *Immunol. Invest. 15*, 123–138.

Allen, H. J., Karakousis, C., Piver, M. S., Gamarra, M., Nava, H., Forsyth, B., Matecki, B., Jazayeri, A., Sucato, D., Kisailus, E., & DiCioccio, R. (1987a) *Tumor Biol. 8*, 218–229.

Allen, H. J., Cywinski, M., Palberg, R., & DiCioccio, R. (1987b) *Arch. Biochem. Biophys. 256*, 523–533.

Barondes, S. H. (1984) *Science 223*, 1259–1264.

Barondes, S. H., Gitt, M. A., Leffler, H., & Cooper, D. N. W. (1988) *Biochimie 70*, 1627–1632.

Carding, S. R., Thorpe, S. J., Thorpe, R., & Feizi, T. (1985) *Biochem. Biophys. Res. Commun. 127*, 680–686.

Catt, J. W., & Harrison, F. L. (1985) *J. Cell Sci. 73*, 347–359.

Cerra, R. F., Gitt, M. A., & Barondes, S. H. (1985) *J. Biol. Chem. 260*, 1074–1077.

Clerch, L. B., Whitney, P., Hass, M., Brew, K., Miller, T., Werner, R., & Massaro, D. (1988) *Biochemistry 27*, 692–699.

Couraud, P.-O., Casentini-Borocz, D., Bringman, T. S., Griffith, J., McGrogan, M., & Nedwin, G. E. (1989) *J. Biol. Chem. 264*, 1310–1316.

Gitt, M. A., & Barondes, S. H. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 7603–7607.

Greenfield, N., & Fosman, G. D. (1969) *Biochemistry 8*, 4108–4116.

Harrison, F. L., & Chesterton, C. J. (1980) *FEBS Lett. 122*, 157–165.

Hirabayashi, J., & Kasai, K. (1984) *Biochem. Biophys. Res. Commun. 122*, 938–944.

Hirabayashi, J., & Kasai, K. (1988) *J. Biochem. (Tokyo) 104*, 1–4.

Hirabayashi, J., Oda, Y., Oohara, T., Yamagata, T., & Kasai, K. (1987a) *Biochim. Biophys. Acta 916*, 321–327.

Hirabayashi, J., Kawasaki, H., Suzuki, K., & Kasai, K. (1987b) *J. Biochem. (Tokyo) 101*, 775–787.

Hirabayashi, J., Kawasaki, H., Suzuki, K., & Kasai, K. (1987c) *J. Biochem. (Tokyo) 101*, 987–995.

Hirabayashi, J., Ayaki, H., Soma, G., & Kasai, K. (1989) *Biochim. Biophys. Acta 1008*, 85–91.

Laemmli, U. (1970) *Nature 227*, 680–685.

Laing, J. G., Robertson, M. W., Gritzmacher, C. A., Wang, J. L., & Liu, F.-T. (1989) *J. Biol. Chem. 264*, 1907–1910.

Lee, R. T., Ichikawa, Y., Allen, H. J., & Lee, Y. C. (1990) *J. Biol. Chem.* (in press).

Mahony, W. C., Smith, P. K., & Hermondson, M. A. (1981) *Biochemistry 20*, 443–448.

Matsudaira, P. (1987) *J. Biol. Chem. 262*, 10035–10038.

Mecham, R. P., Hinek, A., Entwistle, R., Wrenn, D. S., Griffin, G. L., & Senior, R. M. (1989) *Biochemistry 28*, 3716–3722.

Monsigny, M., Roche, A.-C., Kieda, C., Midoux, P., & Obrenovitch, A. (1988) *Biochimie 70*, 1633–1649.

Nishikawa, K., Kubota, Y., & Ooi, T. (1983) *J. Biochem. (Tokyo) 94*, 997–1007.

Ohyama, Y., & Kasai, K. (1988) *J. Biochem. (Tokyo) 104*, 173–177.

Paroutaud, P., Levi, G., Teichberg, V. I., & Strosberg, A. D. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6345–6348.

Raz, A., Pazerini, G., & Carmi, P. (1989) *Cancer Res. 49*, 3489–3493.

Scott, M. G., Crimmins, D. L., McCourt, D. W., Tarrand, J. J., Eyerman, M. C., & Nahm, M. H. (1988) *Biochem. Biophys. Res. Commun. 155*, 1353–1359.

Southan, C., Aitken, A., Childs, R. A., Abbott, W. M., & Feizi, T. (1987) *FEBS Lett. 214*, 301–304.

Sparrow, C. P., Leffler, H., & Barondes, S. H. (1987) *J. Biol. Chem. 262*, 7383–7390.

Wilson, T. J. G., Firth, M. N., Powell, J. T., & Harrison, F. L. (1989) *Biochem. J. 261*, 847–852.