of the Endocrine Society, Seattle, WA (Abstr. 1523).
Leung, D. W., Spencer, S. A., Cachianes, G., Hammonds, R. G., Collins, C., Henzel, W. J., Barnard, R., Waters, M. J., & Wood, W. I. (1987) *Nature 330*, 537-543.
Li, C. H., & Evans, H. M. (1944) *Science 99*, 183-184.
Li, C. H., & Liu, W. R. (1964) *Experientia 20*, 169-178.
Means, G. E., & Feeney, R. E. (1971) in *Chemical Modifications of Proteins*, Holden-Day Inc., San Francisco, CA.
Nicoll, C. S., Mayer, G. L., & Russell, S. H. (1986) *Endocr. Rev. 7*, 169-203.
Noso, T., Yasuda, A., Kawazone, I., Takehara, H., Takahashi, A., Sakai, K., & Kawauchi, H. (1988) *Int. J. Pept. Protein Res. 32*, 579-589.
Schuler, L. A., Shimomura, K., Kessler, M. A., Zieler, C. G., & Bremel, R. D. (1988) *Biochemistry 27*, 8443-8448.
Shechter, Y., Schlessinger, J., Jacobs, S., Chang, K.-J., & Cuatrecasas, P. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2135-2139.
Sluyterman, L. A. E. (1968) *Biochim. Biophys. Acta 151*, 178-187.
Stark, G. R. (1965) *Biochemistry 4*, 1030-1036.
Tanaka, T., Shiu, R. P. C., Gout, P. W., Beer, C. T., Noble, R. L., & Friesen, H. G. (1980) *J. Clin. Endocrinol. Metab. 51*, 1058-1063.
Uchida, E., Shimokawa, S., Nishida, Y., Takasu, H., Ikehara, M., Uesugi, S., Tomita, K., Tanaka, A., Morikawa, M., Hayakawa, T., & Nishikawa, S. (1990) *Biochem. Biophys. Res. Commun. 172*, 357-363.

Synthesis, Isolation, and Characterization of Endogenous β-Galactoside-Binding Lectins in Human Leukocytes[†,‡]

Howard J. Allen,[*,§] Sally Gottstine,[§,∥] Ashu Sharma,[§] Richard A. DiCioccio,[⊥] Richard T. Swank,[#] and Hua Li[#,○]

Departments of Surgical Oncology, Gynecologic Oncology, and Cell and Molecular Biology, Roswell Park Cancer Institute, Buffalo, New York 14263, and Biology Department, Canisius College, Buffalo, New York 14208

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ABSTRACT: Galaptin, a β-galactoside-binding lectin, was isolated from human buffy coat cells (peripheral leukocytes) and spleen by affinity chromatography. The molecular weight (32K) of the native buffy coat galaptin was similar to that for splenic galaptin. Their subunit molecular weight (14.5K), p*I* (4.60–4.85), and amino acid composition were identical. Both galaptins showed the presence of a single polypeptide when subjected to reversed-phase HPLC. Monospecific rabbit polyclonal antiserum raised against the 14.5-kDa subunit of splenic galaptin reacted with a 14.5-kDa polypeptide present in buffy coat cells, Epstein–Barr virus-immortalized B lymphoblastoid cells, and HL-60 promyelocytic leukemia cells. However, galaptin was not synthesized in vitro by buffy coat cells. Rather, a monomeric β-galactoside-binding protein of M_r 15.5–16.5K that is immunologically distinct from galaptin was synthesized. This galactoside-binding protein was separable from galaptin by polyacrylamide gel electrophoresis and by anion-exchange chromatography. In contrast, immunoprecipitation experiments confirmed that galaptin was synthesized by the B lymphoblastoid cells. cDNA corresponding to the B lymphoblastoid cell mRNA encoding galaptin was amplified by the polymerase chain reaction. The amplified product was partially sequenced, and 299 nucleotides were identified. The derived amino acids corresponded to residues 6–65, 84–114, and 118–126 found to be present in human splenic galaptin. Immunohistochemical analyses revealed that galaptin was distributed throughout the cytoplasm of B lymphoblastoid cells rather than being localized to the cell surface. The results presented here demonstrate that galaptin is present in a variety of leukocytes including buffy coat cells. Although buffy coat cells may accumulate galaptin, they do not synthesize it in vitro. The 15.5–16.5-kDa β-galactoside-binding lectin that is synthesized does not appear to accumulate in the buffy coat cells, and it may be a secretory protein [Allen, H. J., et al. (1986) *Immunol. Invest.* 15, 123–138].

The S-type lectins (Drickamer, 1989) are soluble, generally cation-independent, thiol-dependent proteins and are found in a wide variety of tissues and cells (Barondes et al., 1988). Studies of the physicochemistry of the S-type lectins have revealed a variety of expressed proteins with *N*-acetyllactosamine-binding specificity. The major S-type lectin that is frequently isolated from mammalian sources consists of a 30-kDa dimer composed of identical subunits (Allen et al., 1987a,b). In some cases, the monomer may be the primary form isolated (Merkle et al., 1989). For convenience, we have chosen to refer to this particular S-type lectin as galaptin. This is a more narrow definition than that originally stated (Harrison & Chesterton, 1980).

The amino acid sequence of galaptin of human origin has been directly determined (Hirabayashi & Kasai, 1988; Sharma et al., 1990). The gene coding for human galaptin has been cloned from cDNA from different sources, and it has been

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* Address correspondence to this author.
[§] Department of Surgical Oncology, Roswell Park Cancer Institute.
[∥] Biology Department, Canisius College.
[⊥] Department of Gynecologic Oncology, Roswell Park Cancer Institute.
[#] Department of Cell and Molecular Biology, Roswell Park Cancer Institute.
[○] Present address: Frederick Cancer Research Facility, Frederick, MD 21701.

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sequenced (Abbott & Feizi, 1989; Couraud et al., 1989; Hirabayashi et al., 1989). The cloning data revealed the presence of a single gene coding for the galaptin monomer, in contrast to previous expectations (Gitt & Barondes, 1986). The genomic sequence and organization of the human galaptin gene and a related lectin gene have now been reported (Gitt & Barondes, 1991). Four exons are present in the galaptin gene, and several potential regulatory sequences are located upstream.

The galaptin polypeptide may also occur as part of what appear to be fusion proteins perhaps derived from rearranged genes. One of the striking examples of this occurs in the rodent system and is variously called metastasis-associated lectin (Raz et al., 1989), CBP-35 (Laing et al., 1989), low-affinity IgE receptor (ε-BP) (Frigeri et al., 1990), Mac-2 antigen (Cherayil et al., 1989), and low molecular weight non-integrin laminin receptor (Woo et al., 1990). All of these proteins have now been found to be identical or very similar. A corresponding relationship appears to be present in humans (Cherayil et al., 1990; Robertson et al., 1990). The carboxyl half of these molecules contains the region homologous to galaptin. The amino-terminus region is characterized by the presence of collagen-like sequences.

A variety of other S-type lectins have been reported (Barondes et al., 1988; Sparrow et al., 1987; Leffler et al., 1989). The relationship of these lectins to others which have been characterized is not always readily apparent. Some of the polypeptides appear to be degradation fragments (Leffler et al., 1989). Some others (Carding et al., 1985) may be related to the propensity of galaptin to form very stable aggregates (unpublished observations). The immunodetection of other apparent S-type lectins may be due to the presence of a common epitope in unrelated proteins (Abbott et al., 1989).

The synthesis and secretion of S-type lectins by leukocytes are of interest because lactosaminoglycans present at the leukocyte cell surface may be physiologically significant lectin receptors that could mediate autocrine or paracrine functions. Previous studies showed that fresh human buffy coat cells in vitro synthesize a ≈15.5-kDa monomeric lactose-binding carbohydrate-binding protein (CBP)[1] (Allen, 1986; Allen et al., 1986) whereas HL-60 cells synthesize a galaptin-like dimeric lectin in addition to a monomeric lectin of molecular mass different from CBP. However, it was not known if buffy coat cells contained dimeric galaptin nor if the metabolically labeled CBP was monomeric galaptin.

The data presented here show that buffy coat cells accumulate galaptin but apparently not CBP. In contrast, buffy coat cells synthesize in vitro CBP but little or no galaptin whereas EBV-immortalized B lymphoblastoid cells do synthesize galaptin.

Materials and Methods

Isolation and Analysis of β-Galactoside-Binding Lectins. Spleens were obtained from splenectomy patients and stored at −20 °C until used. Crude buffy coat cells in plasma were purchased from the American Red Cross, Buffalo, NY. Buffy coat cells either were further purified by dextran sedimentation of RBC (4 mL of 5% dextran T-500/0.9% NaCl was mixed with 10 mL of buffy coat cells in plasma and allowed to stand at room temperature for 1–2 h) or were washed immediately with 0.9% NaCl. In some cases, acetone powders were prepared (Allen et al., 1987a; Sharma et al., 1990) prior to extraction of spleen and buffy coat cells.

For in vitro lectin synthesis by buffy coat cells, crude buffy coat cells were purchased from the American Red Cross and further purified by dextran sedimentation to remove RBC. The washed buffy coat cells were incubated with [^{3}H]leucine as previously described (Allen et al., 1986) or were incubated with [^{3}H]leucine (10 μCi/mL)/[^{35}S]methionine (10 μCi/mL) for 48 h in serum-free medium at 9×10^6 cells/mL.

Lectins were isolated by affinity chromatography on asialofetuin–Sepharose as described by Allen et al. (1987a) or Sharma et al. (1990). Galaptin was adsorbed to DEAE-Sephacel for storage at −20 °C (Sharma et al., 1990). Purity and molecular weight were assessed by FPLC on Superose 12 and SDS–PAGE (Allen et al., 1987a), by Bio Gel P-30 chromatography (Allen et al., 1986), and by RP-HPLC and IEF (Sharma et al., 1990).

Western Blot Analysis. Western blot analyses were carried out for extracts of spleen and promyelocytic HL-60 cells, and for purified galaptin from spleen, buffy coat cells, and B lymphoblastoid cells. Conditions of sample preparation, electrophoresis, and immunodetection and the rabbit antisplenic galaptin serum were as previously described (Allen et al., 1990; Sharma et al., 1990).

Immunoprecipitation Experiments. Immunoprecipitation experiments with anti-galaptin serum were carried out with [^{35}S]methionine-labeled EBV-immortalized B lymphoblastoid cells. Cells were pulsed for 1.5 h and then chased for 21 h. The B142 cell line used here and all other details were essentially as given previously (DiCioccio & Brown, 1988). For the experiments reported here, all solutions contained 50 mM lactose. Samples were preclarified by incubation with 15 μL of preimmune serum prior to addition of 15 μL of anti-galaptin serum. This amount of antiserum achieved maximal precipitation of radiolabeled galaptin. Culture media samples were supplemented with 0.1 μg of galaptin to facilitate immunoprecipitation.

Comparative immunoprecipitation and DEAE-Sephacel chromatography experiments were carried out with splenic galaptin radiolabeled by alkylation with [^{14}C]iodoacetamide (Allen et al., 1990) and buffy coat cell CBP metabolically labeled in vitro with [^{35}S]methionine/[^{3}H]leucine. Immunoprecipitation reactions contained 1 μg of galaptin (1000 cpm) or CBP equivalent to 6000 cpm. For this set of experiments, galaptin and CBP were isolated on lactose–Sepharose (Allen & Johnson, 1977) followed by DEAE-Sephacel chromatography.

For all SDS–PAGE experiments, electrophoresis was run until the bromophenol tracking dye reached the gel front. This dye migrated well ahead of the 14.4-kDa standard and unknowns.

Immunocytochemistry. The immunolocalization of galaptin in B142 cells was carried out as described for cells present in patient effusions (Allen et al., 1991). Diaminobenzidine was used to visualize immunobound peroxidase.

cDNA Synthesis and Nucleotide Sequence Analysis of B Lymphoblastoid Cell Galaptin. Total RNA was isolated from 10^8 B142 lymphoblastoid cells by a guanidinium isothiocyanate/CsCl method essentially as described by Ausubel et al. (1988). RNA was quantitated by UV spectrometry (A_{260}/A_{280}). Absence of degradation of intact 28S and 18s rRNA was confirmed by electrophoresis on 1.5% agarose/formaldehyde gels (Maniatis et al., 1982).

Oligonucleotide primers for cDNA synthesis were prepared in the RPMI Biopolymer Facility on a Model 380 DNA synthesizer (Applied Biosystems) and were purified on OPC cartridges (Applied Biosystems). The nucleotide sequence of

[1] Abbreviations: CBP, carbohydrate-binding protein; EBV, Epstein–Barr virus; RBC, red blood cell(s).

the primers was based on the cDNA sequence for human hepatoma galaptin (Abbott & Feizi, 1989). A 20-mer forward primer (GP-20) was based upon cDNA residues −28 to −8 upstream of the adenine of the initiation codon for methionine. A 19-mer reverse primer (GP-19) was complementary to cDNA residues 414-432 at the 3′ end.

First-strand cDNA synthesis primed with GP-19 was carried out by using AMV reverse transcriptase (BRL, Bethesda, MD) essentially as described by Abu-Hadid et al. (1988). Asymmetric amplification of cDNA was carried out essentially as described by Dicker et al., (1989). Thermal cycling was done in a Perkin-Elmer–Cetus thermal cycler (Norwalk, CN). Prior to the addition of *Taq*I polymerase, the reaction mixture was heated at 95 °C for 10 min followed by incubation at 60 °C for 2 min. After enzyme addition, incubation proceeded for 5 min at 70 °C. The mixture was then subjected to 30 cycles of amplification: denaturation at 95 °C for 2 min; annealing at 60 °C for 2 min; synthesis at 70 °C for 5 min. The amplification product was purified by centrifugal ultrafiltration. The amplified cDNA was analyzed by electrophoresis on 2% agarose followed by blotting onto nitrocellulose and probing with radiolabeled GP-20 and GP-19 prepared by 5′ end labeling with [γ-^{32}P]ATP using T-4 polynucleotide kinase (BRL, Gaithersburg, MD) essentially as described by Gyllensten and Ehrlich (1988).

Nucleotide sequencing of purified DNA was carried out by the dideoxy chain termination method using Sequenase version 2.0 (U.S. Biochemicals, Cleveland, OH) following the manufacturer's protocol. [α-^{32}P]dATP or [^{35}S]dATPαS was used in the labeling step.

Amino acid analyses were carried out in the Biopolymer Facility, RPCI, using a Waters Pico-Tag system (Waters Division, Milford, MA) following the manufacturer's protocols for precolumn PTH-amino acid derivatization. Samples were hydrolyzed for 24 h at 115 °C in 6 N HCl/phenol vapors.

L-[^{35}S]Methionine (1125 Ci/mmol), [γ-^{32}P]ATP (6000 Ci/mmol), and [^{35}S]dATPαS (1000–1500 Ci/mmol) were obtained from NEN, Boston, MA. [1-^{14}C]Iodoacetamide (17.9 mCi/mmol) and [2,3,4,5-^{3}H]leucine (115 Ci/mmol) were obtained from ICN, Irvine, CA. [α-^{32}P]dATP, 400 Ci/mmol, was obtained from Amersham, Arlington Heights, IL. Electrophoresis and gel filtration calibration proteins were purchased from Sigma, St. Louis, MO. Dextran T-500, Superose 12, and DEAE-Sephacel were products of Pharmacia–LKB, Piscataway, NJ. HL-60 cells were provided by Dr. E. Kisailus, Canisius College, Buffalo, NY.

Results

To obtain a sufficient quantity of CBP for analysis, 10–20 units of buffy coat cells from different individuals were pooled. A portion of the pool was taken for metabolic labeling of CBP with [^{3}H]leucine. The remainder was extracted for CBP isolation. The radiolabeled extract was mixed with the unlabeled extract prior to affinity chromatography. Aliquots of the lactose-eluted fractions were analyzed by SDS–PAGE. A single polypeptide of M_r 14.5K, detectable by Coomassie blue staining after SDS–PAGE, was eluted with lactose (Figure 1A). This polypeptide had the same electrophoretic mobility as splenic galaptin (Figure 1B). Silver staining of gels occasionally showed the presence of a minor band at ≈30 kDa in the lactose-eluted buffy coat cell CBP preparation (Figure 1B).

The radioactive CBP fractions eluted from the affinity column were pooled. Aliquots were analyzed by gel filtration on Superose 12 to determine the native protein molecular weight (Figure 2A) and on BioGel P-30 to determine the radioactive CBP molecular weight (Figure 2B). Protein detectable by A_{280nm} eluted from Superose 12 at M_r 32K but not at a position corresponding to a putative monomer. In contrast, metabolically labeled CBP eluted from P-30 at M_r 16.5K with a small peak of radioactivity present at ≈25 kDa. No radioactivity eluted corresponding to the 32-kDa protein. Similar results were obtained for fresh, 1-day-, and 2-day-old buffy coat cells.

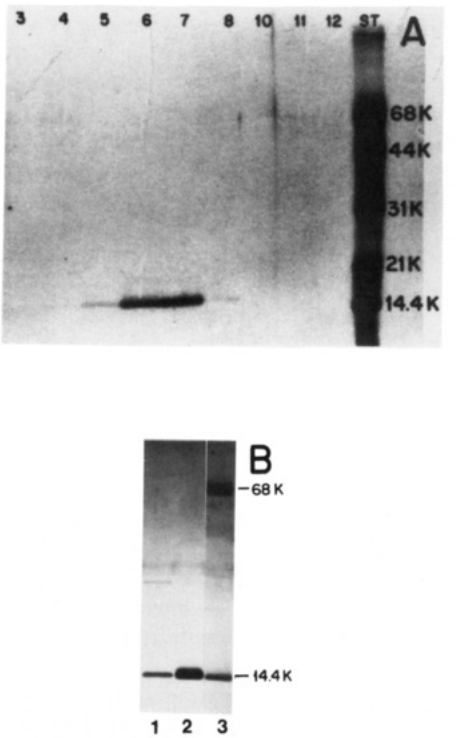

FIGURE 1: (A) SDS–PAGE of lactose-eluted affinity column fractions of buffy coat cell lectin. Aliquots (50 μL) of fractions 3–8 and 10–12 were subjected to SDS–PAGE on 10–20% acrylamide gradient gels followed by staining with Coomassie blue. The affinity column was allowed to sit in 0.1 M lactose for 24 h, 4 °C, between fractions 9 and 10 (9 not analyzed). The molecular weights of protein standards in the end lane are indicated. (B) SDS–PAGE of buffy coat cell lectin and splenic galaptin. Pooled buffy coat cell lectin and splenic galaptin were electrophoresed as above followed by silver staining. Lane 1, buffy coat cell lectin; 2, splenic galaptin; 3, molecular weight standards.

A series of experiments were carried out to determine if the buffy coat cell 32-kDa lectin was related to splenic galaptin and if a similar protein was present in HL-60 cells and human B lymphoblastoid cells.

A single polypeptide which eluted at 44 min was detected by reverse-phase HPLC for splenic galaptin and for the affinity-purified protein from buffy coat cells (Figure 3A). IEF revealed the presence of three distinct bands corresonding to pI's of 4.60, 4.80, and 4.85 for galaptin and buffy coat cell protein (Figure 3B). The staining density of the more acidic bands increased as lectin solutions aged.

Amino acid analyses were carried out on splenic galaptin and buffy coat cell protein. The results are summarized in Table I. The two proteins were found to have virtually identical amino acid compositions. Attempts at peptide sequencing with an Applied Biosystems 470A gas-phase sequencer showed that the amino terminus for both proteins was blocked.

Western blot analyses using anti-[splenic galaptin] serum revealed the presence of an immunoreactive polypeptide of 14.5 kDa in extracts of spleen and HL-60 cells (Figure 4A). A similar polypeptide was immunodetected for the asialofetuin-binding protein purified from buffy coat and B lymphoblastoid cells (Figure 4A). Immunoprecipitation experiments with [^{35}S]methionine-labeled B lymphoblastoid cell

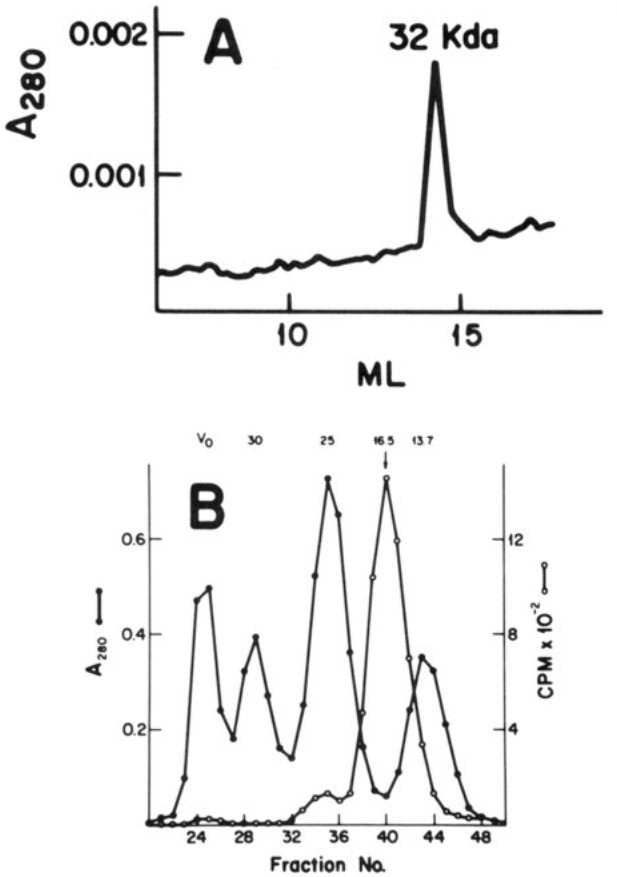

FIGURE 2: (A) Superose 12 chromatography of buffy coat cell lectin. The sample was loaded via a 100-μL injection loop onto a 1 × 24 cm Superose 12 column equilibrated with 0.1 M NaCl/0.05 M PO_4/0.002 M EDTA/0.01 M lactose/0.05% NaN_3, pH 7.3. Flow rate = 0.5 mL/min. (B) BioGel P-30 chromatography of radiolabeled buffy coat cell CBP. An aliquot of metabolically radiolabeled CBP was mixed with calibration proteins and loaded on the column with ascending flow. Two 0.9 × 60 cm columns were connected in tandem and equilibrated with 0.15 M NH_4HCO_3, pH 7.3. Fractions were collected in scintillation vials. After determination of A_{280nm}, the fractions were counted for radioactivity. Flow rate = 4.8 mL/h. Fraction volume = 1.0 mL. Load volume = 1.0 mL; V_0 = void volume. The elution positions of calibration standards and CBP are indicated.

Table I: Amino Acid Composition of Galaptin Isolated from Spleen and Buffy Coat Cells

	mol of AA/mol of monomer			mol of AA/mol of monomer	
AA	spleen[a]	buffy coat cells	AA	spleen[a]	buffy coat cells
Asx	22	22	Tyr	2	2
Glx	11	10	Val	9	9
Ser	5	6	Met	1	1
Gly	12	12	Cys[b]	3	2
His	2	2	Ile	3	4
Arg	5	5	Leu	12	13
Thr	4	4	Phe	10	11
Ala	16	16	Lys	7	8
Pro	8	9	Trp	*c*	*c*

[a] From Sharma et al. (1990). [b] Values are estimates. [c] Not determined.

extracts and culture media showed that these cells synthesize the immunoreactive polypeptide of 14.5 kDa (Figure 4B). Some of the polypeptide was detected in the medium after 21 h of chase.

To directly compare the B lymphoblastoid cell lectin with splenic galaptin, experiments were carried out to derive the amino acid sequence of the B lymphoblastoid cell protein via nucleotide sequencing of amplified cDNA. The cultured B

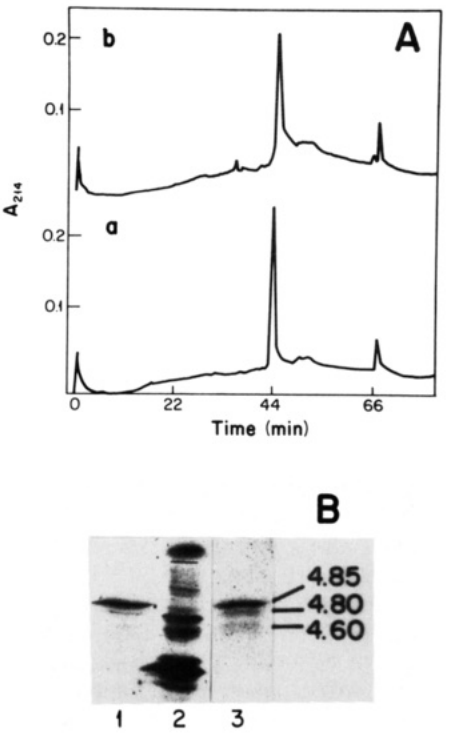

FIGURE 3: (A) Reverse-phase HPLC of reduced and carboxymethylated splenic galaptin and buffy coat cell lectin. Samples were loaded on an Aquapore RP-300 column and eluted as described under Materials and Methods. (a) Splenic galaptin; (b) buffy coat cell lectin. (B) IEF of splenic galaptin and buffy coat cell lectin. Isoelectric focusing was carried out on a pH 4–6.5 IEF Phastgel. The gel was silver stained. The p*I* of the observed polypeptides is indicated. (1) Buffy coat cell lectin; (2) standards; (3) splenic galaptin.

cells were chosen for these experiments because they are a more convenient source of high-quality purified RNA relative to the buffy coat cells. Primers were constructed on the basis of the 3′- and 5′-termini nucleotide sequences of hepatoma galaptin (Abbott & Feizi, 1989). The resulting amplified DNA corresponded to 460 bp, equivalent to a complete galaptin subunit polypeptide. The results of hybridization experiments with radiolabeled primers as probes confirmed that asymmetric PCR resulted in an amplification of the desired ssDNA. The partial nucleotide sequence of this DNA and the derived amino acid sequence are shown in Figure 5. These correspond to amino acid residues 6–65, 84–114, and 118–126 of hepatoma galaptin, and they are identical with those of splenic galaptin (Sharma et al., 1990).

The B lymphoblastoid cell galaptin is not exclusively localized to the cell surface but is distributed throughout the cytoplasm (not shown) as is the case for many cell types (Allen et al., 1991). Attempts at immunostaining of buffy coat cell sections have not been successful due to loss of cell sections from the slides during processing.

A series of immunoprecipitation and DEAE-Sephacel chromatography experiments were carried out to determine if the metabolically labeled buffy coat cell CBP was related to the splenic galaptin monomer. The results are summarized in Figure 6 and show that (a) buffy coat cell CBP does not bind to DEAE-Sephacel under conditions that permit complete binding of galaptin (lanes 1, 2, 6, 8, and 9), (b) buffy coat cell CBP is not precipitated by antisplenic galaptin serum under conditions that immunoprecipitated >75% of galaptin (lanes 3, 4, 5, 7, and 10), and (c) buffy coat CBP is distinguished from the galaptin monomer by electrophoresis of the reduced and alkylated proteins (lanes 5, 6, 9, and 10). These results were quite unexpected and show that galaptin and buffy coat

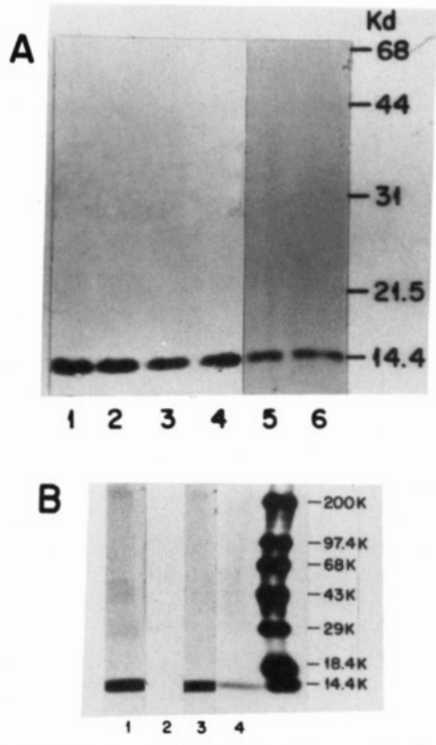

FIGURE 4: (A) Western blot analysis of tissue extracts and purified galaptin. The blots were probed with anti-[splenic galaptin]. Calibration markers are indicated: (1) 10 ng of splenic galaptin; (2) 10 ng of buffy coat cell lectin; (3) 5 ng of B lymphoblastoid cell lectin; (4) hairy cell leukemic spleen extract; (5) HL-60 cell extract; (6) 10 ng of splenic galaptin. (B) Immunoprecipitation of galaptin from B142 lymphoblastoid cells with anti-[splenic galaptin] serum. The cells were labeled with [^{35}S]methionine and then placed in chase media. The immunoprecipitates were electrophoresed on 2.5–27% acrylamide gradient gels, and the radioactive polypeptides were visualized by fluorography. The molecular weights of calibration standards are indicated. Lanes 1 and 2, immunoprecipitates of cells and medium, respectively, after 8 h of chase; lanes 3 and 4 immunoprecipitates of cells and medium, respectively, after 21 h of chase.

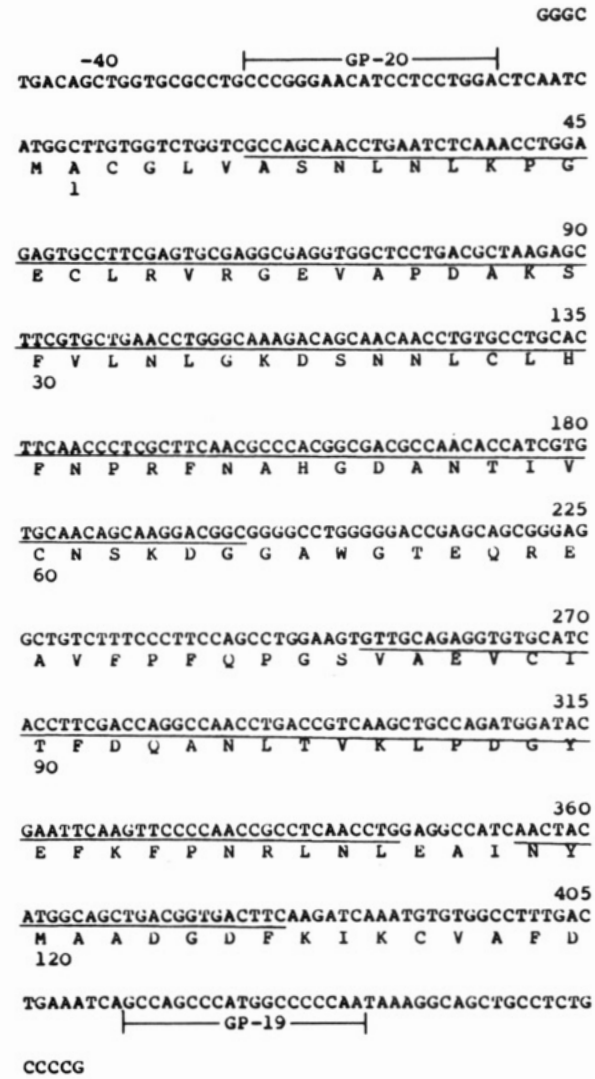

FIGURE 5: Partial nucleotide and deduced amino acid sequence of B lymphoblastoid cell galaptin. The complete sequences and numbering shown are for hepatoma galaptin (Abbott & Feizi, 1989). The nucleotide sequence is numbered, and the adenine of the initiating methionine is designated as "1". The derived amino acid sequence is also numbered with the first alanine residue designated as "1". GP-20 and GP-19 are the forward and reverse primers used here. The partial sequences determined by us for B lymphoblastoid cell galaptin are indicated by the underline.

cell CBP are distinctly different molecules rather than CBP being monomeric galaptin.

Discussion

Leukocytes are known to contain a variety of lectins of generally unknown functions (Monsigny et al., 1988). Many of the identified lectins have a galactoside-binding specificity. Multiple proteins immunologically related to the β-galactoside-binding lectin, galaptin, were reported to be present in human lymphoid cells (Carding et al., 1985). However, rabbit antiserum raised against the human splenic galaptin monomer reacted with only a single polypeptide of M_r 14.5K present in mammalian splenic (Sharma et al., 1990) and human tissue (Allen et al., 1990, 1991) extracts.

The studies reported here were carried out to obtain a better understanding of galaptin and its expression in human leucocytes and to determine if the metabolically labeled buffy coat cell lactose-binding CBP previously described (Allen, 1986; Allen et al., 1986) was related to the galaptin monomer.

Despite the fact that buffy coat cells are a very heterogeneous population, they synthesize in vitro a major galactoside-binding monomeric CBP of M_r 15.5–16.5K. Since this lectin was isolated in the presence of EDTA and thiols, and it had a carbohydrate-binding specificity similar to galaptin (Allen, 1986), we came to assume, without proof, that the CBP was monomeric galaptin. This assumption was consistent with the isolation of monomeric galaptin from human placenta (Hirabayashi et al., 1987) and from porcine heart (Merkle et al., 1989). The data reported here demonstrate that buffy coat cells do contain galaptin that can be isolated en masse (25–50 μg/unit of buffy coat cells). The buffy coat galaptin was isolated as a dimer, however. The dimeric galaptin of buffy coat cells appears to be identical with splenic galaptin (Sharma et al., 1990). Their native and monomeric molecular weights are the same; they yield similar peptide patterns upon IEF; they have identical amino acid composition; they are immunologically cross-reactive; they both have blocked amino termini; and they behave similarly on reverse-phase HPLC. Also, V8 protease digestion of splenic and buffy coat galaptin yielded identical peptide patterns as revealed by SDS–PAGE (not shown).

Rabbit antiserum raised against the 14.5-kDa splenic galaptin subunit appears to be monospecific (Sharma et al., 1990; Allen et al., 1990, 1991). With adequate reduction and alkylation of samples, this antiserum reacted with only a 14.5-kDa polypeptide present in splenic and HL-60 cell extracts and also reacted with galaptin purified from buffy coat and B lymphoblastoid cells. No reactivity was observed corre-

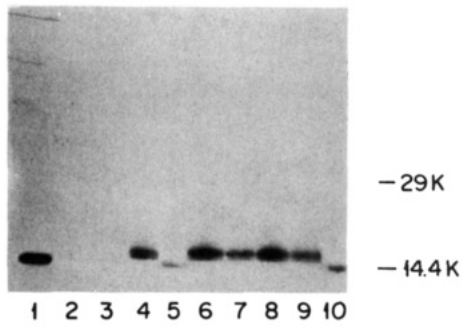

FIGURE 6: DEAE-Sephacel chromatography and immunoprecipitation with anti-[splenic galaptin] serum of metabolically radiolabeled buffy coat cell CBP and ^{14}C-alkylated splenic galaptin. Starting galaptin and CBP were isolated by affinity chromatography on lactose–Sepharose in the presence of 0.01 M mercaptoethanol/0.001 M EDTA. Splenic galaptin was further purified by adsorption of the lactose eluate to 0.5 mL of DEAE-Sephacel equilibrated with 0.02 M NaCl/0.01 M Tris, pH 7.5. After the DEAE-Sephacel column was washed with equilibration buffer, the galaptin was eluted with 0.2 M NaCl/0.01 M Tris, pH 7.5, and was radiolabeled as described under Materials and Methods. The lactose-eluted metabolically labeled buffy coat cell CBP was similarly chromatographed on DEAE-Sephacel. Samples were reduced and alkylated prior to SDS–PAGE on 12.5% acrylamide gels followed by fluorography. Lane 1, galaptin standard; 2, buffy coat cell CBP eluted from DEAE with 0.2 M NaCl; 3, immunoprecipitate of buffy coat cell CBP present in 0.02 M NaCl DEAE wash-through; 4 and 7, supernate of buffy coat cell CBP (lactose eluate) after precipitation with anti-galaptin serum; 5 and 10, immunoprecipitate of galaptin; 6, 8, and 9, buffy coat cell CBP present in 0.02 M NaCl DEAE wash-through. Replicate lanes were loaded with differing amounts of sample.

sponding to the previously reported 18.5-kDa galactoside-binding protein synthesized by HL-60 cells (Allen et al., 1986). Hence, the 18.5-kDa protein present in HL-60 cells is distinct from the 14.5-kDa subunit of galaptin. This is in agreement with the data of Courad et al. (1989).

Immunoprecipitation experiments with anti-[splenic galaptin] serum confirmed the presence of no other than a 14.5-kDa immunoreactive polypeptide present in B142 lymphoblastoid cells. It is worth noting that galaptin was not detected in a sample of peritonitis-elicited mononuclear cells (Allen et al., 1987b). B142 cells may secrete galaptin although the kinetics of secretion have not been characterized. Galaptin is present in the cytoplasm of B142 cells, and the presence of galaptin in the culture medium could be influenced by cell leakage. Recapture of released galaptin via cell receptors may also occur.

Direct amino acid sequence analysis of peptide fragments from human splenic galaptin (Sharma et al., 1990) showed that this galaptin is identical with human placental (Hirabayashi & Kasai, 1988) and human hepatoma (Abbott & Feizi, 1989) galaptin. The partial amino acid sequence of the B lymphoblastoid cell galaptin reported here was obtained via nucleotide sequencing of cDNA. The size of the amplified cDNA corresponded to that expected from the full reading frame and is in agreement with the molecular mass of the galaptin monomer. The derived partial amino acid sequence corresponded to and was identical with residues 6–65, 84–114, and 118–126 of splenic galaptin.

In contrast to expectations, the metabolically labeled buffy coat cell CBP is distinctly different from splenic galaptin. Galaptin binds to DEAE-Sephacel under low salt conditions and can be eluted with 0.2 M NaCl (Sharma et al., 1990). The affinity-purified metabolically labeled CBP does not adsorb to DEAE under those conditions. Labeled CBP is not precipitated by anti-galaptin serum under conditions that precipitate ^{14}C-alkylated splenic galaptin and metabolically labeled B142 cell galaptin. Side-by-side SDS–PAGE of reduced and alkylated samples show a clear separation of splenic galaptin and labeled buffy coat cell CBP. Occasionally, there is some indication that the labeled buffy coat CBP may contain a major and a minor polypeptide of very similar molecular weight (Figure 6, lane 6). A minor galactoside-binding component of $\approx M_r$ 25–30K may also be synthesized by the buffy coat cells.

The CBP does not seem to accumulate in buffy coat cells since we have been unable to detect it in affinity column isolates except by metabolic labeling methods. Hence, relative to galaptin, it is present in a very small amount. This would be consistent with a secretory, rather than a cell-bound, lectin (Allen et al., 1986). The identity of the buffy coat CBP is unknown. Whether it is related to the HL-60 18.5-kDa protein or related to one of the multiple S-type galactoside-binding lectins reported to be present in human and rat lung (Sparrow et al., 1987; Cerra et al., 1985) and in rat intestine (Leffler et al., 1989) is unknown. Its role as a putative precursor subunit for some other lectin for which maturation is perturbed as a result of in vitro incubation of buffy coat cells must also be considered.

Although we have demonstrated the presence of galaptin in buffy coat cells, B142 cells, and HL-60 cells and in tissue macrophages (Allen et al., 1991) and activated histiocytes (Allen et al., 1987b), and have demonstrated the synthesis of galaptin by B142 cells, the in vitro synthesis of galaptin by buffy coat cells has not been observed by us. We currently do not know the basis for this observation. It may be that galaptin synthesis is a highly regulated event which becomes perturbed when peripheral leukocytes are placed in short-term culture. Alternatively, buffy coat cell galaptin may be a product of granulocytes for which protein synthesis has been shut down as a consequence of cell maturation. It is also possible that galaptin is present in buffy coat cells as a result of capture and endocytosis of soluble lectin. Further studies are required to understand this observation.

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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.
Abu-Hadid, M. M., Fuji, H., & Sood, A. K. (1988) *Mol. Immunol. 25*, 739–749.
Allen, H. J. (1986) *Immunol. Invest. 15*, 379–392.
Allen, H. J., & Johnson, E. A. Z. (1977) *Carbohydr. Res. 58*, 253–265.
Allen, H. J., Kachurek, D., Bulera, S., & Kisailus, E. (1986) *Immunol. Invest. 15*, 123–138.
Allen, H. J., Cywinski, M., Palmberg, R., & DiCioccio, R. A. (1987a) *Arch. Biochem. Biophys. 256*, 523–533.
Allen, H. J., Karakousis, C., Piver, M. S., Gamarra, M., Nava, H., Forsyth, B., Matecki, B., Jazayeri, A., Sucato, D., Kisailus, E., & DiCioccio, R. (1987b) *Tumor Biol. 8*, 218–229.
Allen, H. J., Sucato, D., Woynarowska, B., Gottstine, S., Sharma, A., & Bernacki, R. (1990) *J. Cell. Biochem. 43*, 43–57.
Allen, H. J., Sucato, D., Gottstine, S., Kisailus, E., Nava, H., Petrelli, N., Castillo, N., & Wilson, D. (1991) *Tumor Biol. 12*, 52–60.

Ausubel, F. M., Brent, R., Kingston, R. E., Moor, D. D., Seidman, J. G., Smith, J. A., & Struhl, K., Eds. (1988) *Current Protocols in Molecular Biology*, pp 4.2.1-4.2.5, Greene Publishing Associates and Wiley-Interscience, John Wiley and Sons, New York.
Barondes, S. H., Gitt, M. A., Leffler, H., & Copper, D. N. W. (1988) *Biochimie 70*, 1627-1632.
Carding, S. R., Childs, R. A., Thorpe, R., Spitz, M., & Feizi, T. (1985) *Biochem. J. 228*, 147-153.
Cerra, R. F., Gitt, M. S., & Barondes, S. H. (1985) *J. Biol. Chem. 260*, 10474-10477.
Cherayil, B. J., Weiner, S. J., & Pillai, S. (1989) *J. Exp. Med. 170*, 1959-1972.
Cherayil, B. J., Chaitovitz, S., Wong, C., & Pillai, S. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 7324-7328.
Couraud, P.-O., Casentini-Borocz, D., Bringman, T. S., Griffith, J., McGrogan, M., & Nedwin, G. E. (1989) *J. Biol. Chem. 264*, 1310-1316.
DiCioccio, R. A., & Brown, K. S. (1988) *Biochem. Genet. 26*, 401-420.
Dicker, A. P., Matthias, V., Adamo, A., Barreda, C., & Berfino, J. R. (198) *BioTechniques 7*, 830-838.
Drickamer, K. (1989) *Ciba Found. Symp. 145*, 45-58.
Frigeri, L. G., Robertson, M. W., & Liu, F.-T. (1990) *J. Biol. Chem. 265*, 20763-20769.
Gitt, M. A., & Barondes, S. H. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 7603-7607.
Gitt, M. A., & Barondes, S. H. (1991) *Biochemistry 30*, 82-89.
Gyllensten, U. B., & Erlich, H. A. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7652-7658.
Hirabayashi, J., & Kasai, K. (1988) *J. Biochem. 104*, 1-4.
Hirabayashi, J., Kawasaki, H., Suzuki, K., & Kasai, K. (1987) *J. Biochem. 101*, 987-995.
Hirabayashi, J., Ayaki, H., Soma, G., & Kasai, K. (1989) *Biochim. Biophys. Acta 1008*, 85-91.
Laing, J. G., Robertson, M. W., Gritzmacher, C. A., Wang, J. L., & Liu, F.-T. (1989) *J. Biol. Chem. 264*, 1907-1910.
Leffler, H., Masiarz, F. R., & Barondes, S. H. (1989) *Biochemistry 28*, 9222-9229.
Maniatis, T., Fritsch, F. E., & Sambrook, J. (1982) *Molecular Cloning. A Laboratory Handbook*, pp 202-203, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
Merkle, R. K., Zhou, Q., Schultz, T. K., Harper, W. B., & Cummings, R. D. (1989) *Arch. Biochem. Biophys. 274*, 404-416.
Monsigny, M., Roche, A.-C., Kieda, C., Midoux, P., & Obrenovitch, A. (1988) *Biochimie 70*, 1633-1649.
Raz, A., Pazerini, G., & Carmi, P. (1989) *Cancer Res. 49*, 3489-3493.
Robertson, M. W., Albrandt, K., Keller, D., & Liu, F. T. (1990) *Biochemistry 29*, 8093-8100.
Sharma, A., Chemelli, R., & Allen, H. J. (1990) *Biochemistry 29*, 5309-5314.
Sparrow, C. P., Leffler, H., & Barondes, S. H. (1987) *J. Biol. Chem. 262*, 7383-7390.
Woo, H.-J., Shaw, L. M., Messier, J. M., & Mercurio, A. M. (1990) *J. Biol. Chem. 265*, 7097-7099.

Enzymatic Redox Chemistry: A Proposed Reaction Pathway for the Six-Electron Reduction of SO_3^{2-} to S^{2-} by the Assimilatory-Type Sulfite Reductase from *Desulfovibrio vulgaris* (Hildenborough)†

Jian Tan and J. A. Cowan*
Evans Laboratory of Chemistry, The Ohio State University, 120 West 18th Avenue, Columbus, Ohio 43210
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ABSTRACT: A detailed reaction pathway for the six-electron reduction of SO_3^{2-} to S^{2-} by the assimilatory-type sulfite reductase (SiR) from *Desulfovibrio vulgaris* (Hildenborough) has been duduced from experiments with ^{35}S-labeled enzyme and the relative reaction rates of nitrogenous subtrates. The ligand bridging the prosthetic $[Fe_4S_4]$-siroheme center is apparently exchanged by $^{35}S^{2-}$ in both oxidized and reduced enzyme. This $^{35}S^{2-}$ label was retained in the course of SO_3^{2-} reduction, implicating substrate binding to the nonbridging axial site of the siroheme. A reaction mechanism is proposed in which SO_3^{2-} binds to Fe^{2+} through the sulfur atom, followed by a series of two-electron reductive cleavages of S-O bonds. Protonation of oxygen facilitates bond cleavage, giving hydroxide as leaving group. The bridge remains intact throughout the course of the reaction, providing an efficient coupling pathway for electron transfer between the cluster and siroheme.

The understanding of enzymatic reduction of low molecular weight substrates (e.g., NO_3^-, NO_2^-, SO_4^{2-}, SO_3^{2-}, O_2, N_2) is of general importance and has been widely studied (Mortenson & Thorneley, 1979; Barber, 1984; Witt et al., 1986; Papa, 1983; Hatefi et al., 1985). However, the molecular mechanisms for these reaction pathways are currently lacking in detail. The biological sulfur cycle provides many examples of this type of chemistry. Several sulfate- and sulfite-reducing systems have been described that either provide a source of reduced sulfur for cellular metabolism (assimilatory reduction) or have involvement in anaerobic respiration (dissimilatory reduction) (Le Gall & Postgate, 1973; Peck & Le Gall, 1982; Siegel, 1975). Reduction of SO_4^{2-} to SO_3^{2-} via an adenosyl phosphosulfate intermediate is catalyzed by a flavoprotein (ATP sulfurylase) and adenylyl phosphosulfate reductase (Le Gall & Postgate, 1973; Peck & Le Gall, 1982; Siegel, 1975). Subsequent six-electron reduction of SO_3^{2-} to S^{2-} is catalyzed by a single enzyme where the siroheme chromophore is re-

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