Soluble Lactose-Binding Vertebrate Lectins: A Growing Family†

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ABSTRACT: Extracts of rat intestine contain nine soluble lactose-binding lectins with subunit molecular weights ranging from 14 500 to 19 000 that were purified by affinity chromatography and ion-exchange chromatography. Two of them are either identical with or closely related to other known rat lectins. A third appears to be the isolated carbohydrate-binding C-terminal domain of a known lectin but lacks the N-terminal domain presumed to mediate a different function. The others have not been described previously. Among them, the major rat intestinal lectin, RI-H, and a related protein, RI-G, have N-terminal amino acid sequences with similarities to sequences found in other known rat lectins. Therefore, these results introduce new members of a growing family of these structurally homologous soluble lactose-binding proteins.

Many vertebrate tissues contain soluble lactose-binding (S-Lac)[1] lectins (Barondes, 1984, 1986; Drickamer, 1988). The best characterized is a dimeric protein with a subunit molecular weight that ranges from about 14 000 to 16 000 in different species. Lectins of this type are developmentally regulated, differentially expressed in different tissues, and concentrated in either the cytoplasm of the cell or the extracellular matrix, depending on the tissue and stage of development (Barondes, 1986).

Several other S-Lac lectins have also been isolated and characterized. For example, in rat lung, lectins with subunit molecular weights of 18 000 and 29 000 have been described (Cerra et al., 1985). Related lectins from human lung (Sparrow et al., 1987) and mouse lung (Crittenden et al., 1984) have also been found, and one with an apparent molecular weight of 35 000, designated CBP-35 (Crittenden et al., 1984; Roff & Wang, 1983), has been examined in detail. The amino acid sequence of its C-terminal portion is homologous to the lectins with subunit molecular weights of 14 000–16 000 described above (Jia & Wang, 1988), but its localization in the nucleus of proliferating 3T3 cells suggests different cellular functions (Moutsatsos et al., 1987; Laing & Wang, 1988). In addition, an S-Lac lectin with a subunit molecular weight of 67 000 has been isolated from bovine chondroblasts and shown to have the properties of an elastin receptor (Hinek et al., 1988). There is also evidence for still other related lectins (Allen et al., 1987; Carding et al., 1985; Hirabayashi et al., 1987; Imamura et al., 1984; Mbamalu & Zalik, 1987; Ohara & Yamagata, 1986; Zalik et al., 1983).

Although these related proteins can be grouped together by their common property of binding to lactose, there is evidence that some can be distinguished on the basis of the binding specificities of their active sites. For example, three S-Lac lectins from rat lung were found to have distinct specificities when tested with a battery of naturally occurring mammalian glycoconjugates (Leffler & Barondes, 1986). Similar results were found with three related lectins from human lung (Sparrow et al., 1987). These findings suggest that the lectins function in nature by interacting with complementary endogenous glycoconjugates. In the case of the elastin receptor, this interaction may be critical for macromolecular assembly of elastin subunits into elastic fibers (Hinek et al., 1988).

Because of an interest in the interaction of S-Lac lectins with tissue glycoconjugates, we undertook a study of these proteins in mammalian intestine, a tissue rich in known glycoconjugate structures (Carlstedt et al., 1985; Bjork et al., 1987; Breimer et al., 1982; Hansson et al., 1982). To our surprise, we found that rat intestine had a much wider assortment of S-Lac lectins than any other tissue previously described. Of these, the predominant intestinal lectin with subunit molecular weight 17 000 is distinct from all those observed previously, although it shows structural homology. These results, therefore, introduce some new members of this growing family of S-Lac lectins united not only by their affinity for lactose, but also by some related structural features.

Experimental Procedures

Materials. Frozen whole rat and mouse intestines (including large and small intestine) were obtained from Pelfreeze, Rogers, AR. Lac–Sepharose was prepared by coupling lactose (Sigma) to Sepharose 4B (Pharmacia, Piscataway, NJ) using divinyl sulfone (Sigma) as described (Porath & Ersson, 1973; Levi & Teichberg, 1981). DEAE-Sepharose CL6B, Cm-Sepharose Fast Flow, and a Superose 12 HR 10/30 column were also from Pharmacia.

Purification of a Mixture of β-Galactoside-Binding Lectins from Intestine. A mixture of intestinal S-Lac lectins was prepared as follows: (a) homogenization of about 150 g of tissue in 300 mL of 75 mM KH_2PO_4/Na_2PO_4, pH 7.2, 75 mM NaCl, 4 mM β-mercaptoethanol, and 2 mM EDTA containing 150 mM lactose; (b) centrifugation at 5000*g* for 30 min and at 100000*g* for 60 min; (c) dialysis of the supernatant for 36 h against several changes of this buffer to remove lactose; (d) affinity chromatography on a 30- or 300-mL column of

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[1] Abbreviations: S-Lac lectins, soluble lactose-binding vertebrate lectins; Lac–Sepharose, lactose conjugated to Sepharose 4B; CBP-35, a mouse carbohydrate-binding protein with apparent subunit M_r 35 000; RL-14.5, RL-18, and RL-29, rat lung lectins with apparent subunit M_r 14 500, 18 000, and 29 000, respectively; RI-A to H, rat intestinal lectins A to H; MI-A to C, mouse intestinal lectins A to C; SDS–PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate; IEF, isoelectric focusing.

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Lac–Sepharose, as indicated. Two-milliliter aliquots of phenylmethanesulfonyl chloride solution (50 mg/mL in methanol) were added before homogenization, after the first centrifugation, and after the second centrifugation. The purification method used here is similar to the one used to isolate lectins from lung (Cerra et al., 1985), except that Lac–Sepharose was used for affinity chromatography instead of asialofetuin–Sepharose. The Lac–Sepharose was cheaper and easier to make, was found to have better lectin-binding capacity, and could be reused for a longer time (Whitney et al., 1985).

Ion-Exchange Chromatography. The lectin mixtures were dialyzed against 5 mM NaCl, 4 mM β-mercaptoethanol, and 10 mM Tris-HCl, pH 7.8, except in one case where the pH was 7.4 and 10 mM lactose was added, as indicated in Figure 2a. They were then fractionated on columns of DEAE-Sepharose CL6B using increasing concentrations of NaCl in the above buffer mixture for elution. Use of buffer with pH 7.4 or 7.8 facilitated the purification of different lectins (see Results). The components of the flow-through from the DEAE-Sepharose columns were further resolved by chromatography on Cm-Sepharose Fast Flow equilibrated in 5 mM KH_2PO_4/Na_2PO_4, pH 7.0, containing 4 mM β-mercaptoethanol and, where indicated, 10 mM lactose, using either increasing concentrations of NaCl for elution or isocratic elution. The pH of the flow-through was adjusted to 7.0 by addition of 250 mM KH_2PO_4/Na_2PO_4, pH 6.8, before it was applied to the column. NaCl concentrations were estimated by using conductivity measurements.

Gel Electrophoresis. SDS–PAGE was done as described (Laemmli, 1970) using a Miniprotean II electrophoresis apparatus (Bio-Rad, Richmond, CA) and silver staining according to Morrisey (1981). Isoelectric focusing was done by using the Phast system (Pharmacia) with commercially available precast gels (IEF 3–9, 4–6.5, and 5–8, Pharmacia) and silver staining according to the manufacturer's instructions. Isoelectric points were determined by comparison of the gel positions of lectin proteins with those of Bio-Rad IEF standards. The samples were loaded in the middle of the pH gradient. In cases in which proteins ran off the pH gradient gels when run to equilibrium, electrophoresis was run for a shorter time (nonequilibrium conditions). The prefocusing conditions (before the proteins were loaded on the gel) were identical for both IEF and the studies done under nonequilibrium conditions.

Molecular Weight Determination. Subunit molecular weights were determined by SDS–PAGE. In order to determine the molecular weights of the native, intact proteins, a freshly prepared mixture of intestinal S-Lac lectins was concentrated by using a Centricon 10 microconcentrator (Amicon Co, Danvers, MA) and chromatographed over a Superose 12 HR 10/30 column (Pharmacia) equilibrated in 5 mM KH_2PO_4/Na_2PO_4, pH 7.4, containing 4 mM β-mercaptoethanol, 10 mM lactose, and 150 mM NaCl. Fractions of 250 μL were collected and analyzed by electrophoresis to identify the lectins eluted at each molecular weight interval. The column was calibrated with bovine serum albumin (M_r 67 000), β-lactoglobulin (M_r 35 000), and cytochrome *c* (M_r 12 400).

Reactivity of Lectins with Antisera. Reactivity of the purified intestinal lectins with antisera raised against three rat lung lectins, RL-14.5, RL-18, and RL-29 (Cerra et al., 1985), was studied by dot blots of serial dilutions of the intestinal lectins or Western blots (Towbin et al., 1979) with a 1/100 dilution of each antiserum. The bound antibodies were detected by reaction with biotinylated anti-rabbit IgG followed by reaction with peroxidase–avidin complexes using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. To increase the chance of detecting the potential presence of intact RL-29 in intestine, a fraction that would be expected to be enriched in this lectin was analyzed by staining a Western blot with anti-RL-29. To prepare this fraction, a total rat intestinal lectin preparation was concentrated with a Centricon 10 microconcentrator (Amicon Co, Danvers, MA), dialyzed, and passed through an HPLC anion-exchange column (Bio-Gel TSK-DEAE-5-PW, 75 × 7.5 mm) equilibrated in 5 mM NaCl/10 mM Tris-HCl, pH 7.4, containing 4 mM β-mercaptoethanol and 10 mM lactose. The flow-through, which would contain RL-29 (Cerra et al., 1985), was collected and used for the Western blot.

Amino Acid Composition and N-Terminal Amino Acid Sequencing. The proteins were degraded by 6 N HCl in vacuo using standard procedures, and the amino acids were derivatized and quantitated by using the Pico-Tag method (Bidlingmeyer et al., 1984). The N-terminal amino acid sequences were obtained either on the underivatized proteins or after reduction and derivatization of cysteines with vinylpyridine (Friedman et al., 1970). Edman degradation was performed on an Applied Biosystems (Foster City, CA) 470A protein sequencer using standard chemistry and programs. The phenylthiohydantoin amino acids were identified by narrow-bore high-performance liquid chromatography on an Applied Biosystems 120A analyzer.

Analysis of Carbohydrate-Binding Specificity. To study the binding specificity of the major lectins, we examined the activity of a series of oligosaccharides in inhibiting the binding of radiolabeled lectin to small volumes of Lac–Sepharose, essentially as described (Leffler & Barondes, 1986), except that Lac–Sepharose was used instead of asialofetuin–Sepharose. The lectins RI-H and MI-C were alkylated with iodoacetamide and labeled with Bolton–Hunter reagent and repurified. The specific radioactivities of the labeled lectins were (cpm/μg) as follows: RI-H, 2.5×10^6; MI-C, 10^7. The binding assay and computations were performed as described previously (Leffler & Barondes, 1986).

Results

Purification of Rat Intestinal Lectins. A mixture of the S-Lac lectins from intestine was isolated by affinity chromatography on Lac–Sepharose. This tissue is rich in these proteins, yielding about 10 mg of lectin mixture from 150 g of frozen whole intestine. This is about 1% of the total soluble protein as estimated by the Bradford protein assay (Bradford, 1976). We define these proteins as lectins simply because of their binding properties, without reference to the classical criterion of cell agglutination activity, as discussed previously (Barondes, 1988).

Three hundred milliliters of Lac–Sepharose was sufficient to bind all the solubilized lectins from 150 g of intestine. When 30-mL columns were used, some components of the lectin mixture bound selectively, whereas the majority of the lectins did not bind. This selective binding facilitated the purification of some lectins in the mixture, as indicated below. None of these lectins bound significantly to underivatized Sepharose.

Individual components of the lectin mixtures were resolved by ion-exchange chromatography (Figure 1). First, the total lectin mixture from intestine was dialyzed against a low-salt buffer and fractionated on DEAE-Sepharose using increasing concentrations of NaCl as eluant (Figure 1a). The flow-through from this column was fractionated on a Cm-Sepharose column (Figure 1b). Fraction A from the DEAE-Sepharose

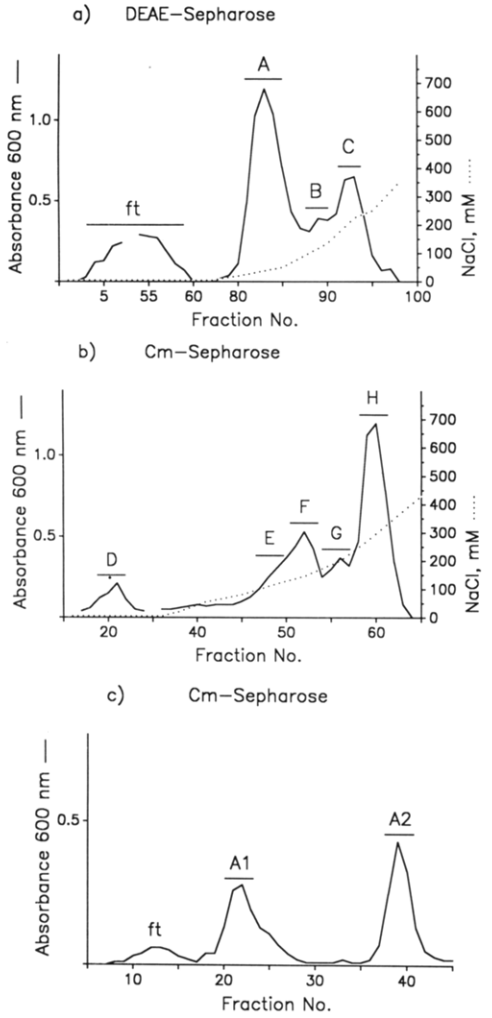

FIGURE 1: Ion-exchange chromatography of rat intestinal lectins. The lectin mixture derived by affinity chromatography on a 300-mL Lac–Sepharose column of extract from 150 g of rat intestines was fractionated further as follows: (a) The lectin mixture was dialyzed against low-salt buffer (5 mM NaCl/10 mM Tris-HCl, pH 7.8) and applied to a DEAE-Sepharose column (1.5 × 30 cm, 50 mL) equilibrated in the same buffer and eluted by increasing concentrations of NaCl. Fractions of 4.25 mL were collected. (b) The pH of the flow-through (ft) from (a) was adjusted to 7.0 by addition of 0.25 M KH_2PO_4/Na_2HPO_4, pH 6.8, and applied to a Cm-Sepharose column (1.5 × 80 cm, 140 mL) equilibrated in 5 mM KH_2PO_4/Na_2PO_4, pH 7.0, and eluted with increasing concentrations of NaCl. Fractions of 3.25 mL were collected. (c) Five-milliliter aliquots of fraction A from (a) were applied to the same Cm-Sepharose column used in (b) and eluted isocratically with 5 mM KH_2PO_4/Na_2PO_4, pH 7.0. Fractions of 2.5 mL were collected. The solid line shows the absorbance measured in the dye-binding assay for protein (Bradford, 1976). The dotted line shows the concentration of NaCl as estimated by measurement of conductance. Fractions were pooled as shown by the bars and are labeled A, A1, A2, and B–H. ft means flow-through.

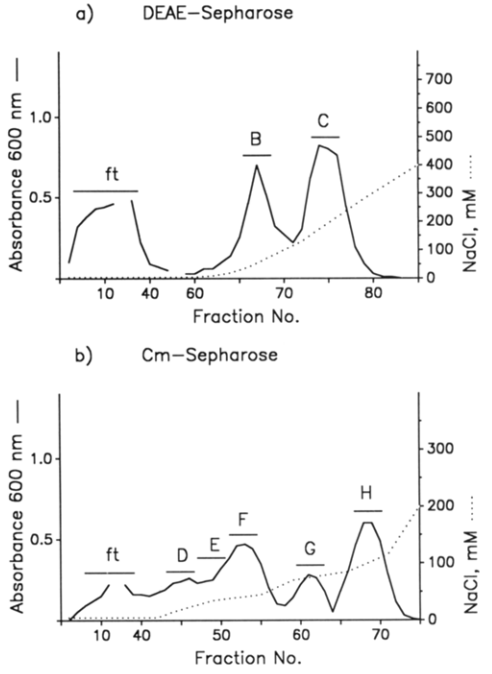

FIGURE 2: Ion-exchange chromatography of rat intestinal lectins using alternative conditions. The starting mixture of lectins was derived from extraction of 150 g of rat intestines and affinity chromatography on a 30-mL Lac–Sepharose column. This column was overloaded with lectin (see text) and selectively enriched in some components. Therefore, fraction B was relatively more abundant, and fraction A was less abundant. The lectins were fractionated by (a) DEAE-Sepharose chromatography followed by (b) Cm-Sepharose chromatography as described in Figure 1a,b except that the pH for the DEAE-Sepharose chromatography was 7.4, smaller columns (1 × 15 cm, 12 mL) were used, the buffers contained 10 mM lactose, and 1-mL fractions were collected. These conditions gave better separation of certain lectins compared with the scheme used in Figure 1. The labeling of fractions is the same as in Figure 1. Thus, fraction B of Figure 1 contained the same lectin as fraction B of Figure 2 etc.

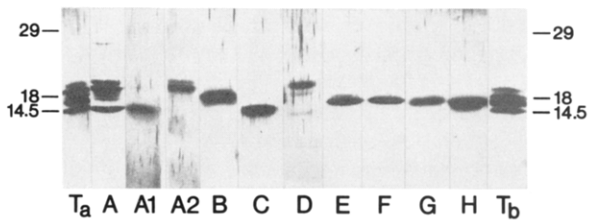

FIGURE 3: SDS–PAGE of rat intestinal lectin fractions. The designations A1, A2, and B–H refer to the fractions isolated by ion-exchange chromatography described in Figures 1 and 2. T are the mixtures of lactose-binding lectins eluted from lactosyl-Sepharose representing a total mixture of intestinal lectins (T_a) or the lectin species that bound preferentially to an overloaded lactosyl-Sepharose column (T_b) (see text); 500–700 ng of the purified fractions and 1000–2000 ng of the mixed fractions (A, T_a, and T_b) were applied to the gel. Three rat lung lectins, RL-14.5, RL-18, and RL-29, were used as molecular weight standards as indicated at the margins.

column was further resolved by chromatography on Cm-Sepharose (Figure 1c). An alternative fractionation scheme was also employed, starting with an eluate from an overloaded Lac–Sepharose column which was enriched in different components followed by a modified scheme for ion-exchange chromatography (Figure 2). With this scheme, much of fraction A was lost, but several others were better resolved (Figure 2).

By combining these purification schemes, nine S-Lac lectins were isolated from extracts of rat intestine. Each was highly purified, although some showed detectable contaminants when examined by SDS–PAGE (Figure 3). In each instance, purity was further evaluated by isoelectric focusing, a procedure which also helped to distinguish lectins with the same subunit molecular weight. Other properties used to characterize the lectins included the molecular weight of the native protein as determined by gel filtration, reactivity with antisera raised against three S-Lac lectins purified from rat lung, N-terminal amino acid sequencing, and amino acid composition. These properties are summarized in Tables I and II but will now be considered in greater detail. The designation rat intestinal lectins A to H is based on the ion-exchange fractionation scheme shown in Figures 1 and 2.

Rat Intestinal Lectins A1 and A2 (RI-A1 and RI-A2). Rat intestinal lectins A1 and A2 were eluted by about 40 mM NaCl from a DEAE-Sepharose column as a mixture together

Table I: Properties of Rat (RI-A to H) and Mouse (MI-A to C) Intestinal Lectins and Rat Lung Lectins (RL-14.5, RL-18, and RL-29)

lectin	yield[a] (μg/g of tissue)	subunit M_r[b] ($\times 10^{-3}$)	no. of subunits[c]	pI[d]	react. with antisera (potency)[e]	N-terminus[f]
RI-A1	6	14.5	1	6.3	anti-RL-14.5 (0.1)	blocked
RI-A2	9	19	1	6.1	none	blocked
RI-B	2	18	1	5.1	anti-RL-18 (1)	free
RI-C	7	14.5	2	4.7	anti-RL-14.5 (1)	nt
RI-D	1	19	1	>9	anti-RL-18 (0.1)	nt
RI-E	1	17	1	6.8	anti-RL-29 (0.2)	nt
RI-F	4	17.5	1	>9	anti-RL-29 (0.5)	free
RI-G	2	17	1	>9	none	free
RI-H	18	17	1	>9	none	free
MI-A	4	19, 20	nt	5.4, 6.1	none	nt
MI-B	6	14.5	nt	5.0, 5.2	anti-RL-14.5 (1)	nt
MI-C	11	17.5, 19	nt	>9	anti-RL-29 (0.05)	free
RL-14.5[g]	12	14.5	2	4.7	anti-RL-14.5 (1)	blocked
RL-18[g]	9	18	1	5.1	anti-RL-18 (1)	blocked
RL-29[g]	4	29	1	>9	anti-RL-29 (1)	blocked

[a] The approximate yield from 150 g of tissue was estimated by the Bradford protein assay. Figures are rounded to the nearest integer. [b] The subunit molecular weight was estimated by SDS–PAGE under reducing conditions (Figures 3 and 7). [c] The number of subunits was estimated from the molecular weight of the native protein as determined by gel filtration. [d] The isoelectric point of the major component(s) in each fraction is shown. [e] Reactivity of the proteins with antisera was determined by dot blotting of serial dilutions of the lectins (see Experimental Procedures). The relative potency was calculated as the smallest amount of intestinal lectin giving a clearly positive reaction divided by the smallest amount of the reference lung lectin (e.g., RL-14.5 for anti-RL-14.5 etc.) giving the same reaction. Thus, a potency of 1 indicated the same reactivity as the reference lectin while lower figures indicate lower reactivity. Reactivities at less than 0.05 are not shown. nt means not tested. [f] Free means available for reaction with phenyl isothiocyanate in the automated Edman procedure. The sequences that could be identified are shown in Figure 4. nt means not tested. [g] For the lung lectins, the data on the molecular weight and subunits are from Cerra et al. (1985). The blocked N-terminus of RL-14.5 is as reported (Clerch et al., 1988). The other data on RL-14.5, RL-18, and RL-29 were determined as part of this investigation.

Table II: Number of Amino Acid Residues per Molecule of Some Intestinal Lectins[a]

amino acid	RI-A1	RI-A2	RI-B	RI-F	RI-G	RI-H
Asx	17	17	17	22	18	18
Thr	7	6	7	10	7	9
Ser	11	7	14	11	8	8
Glx	14	22	23	16	15	11
Pro	5	14	8	7	10	11
Gly	11	19	12	9	26	19
Ala	1	9	11	10	9	8
Val	6	14	10	7	10	10
Ile	4	7	5	9	11	13
Leu	13	11	12	15	11	10
Tyr	3	8	7	6	5	6
Phe	9	13	9	6	13	15
His	5	7	3	3	3	3
Lys	8	7	7	*b*	6	7
Arg	4	7	14	4	10	11
Met	3	4	3	2	3	4

[a] The estimated number of residues per molecule was calculated by using the amino acid composition and the subunit molecular weight. Tryptophan and cysteine were not measured. [b] This determination of Lys was uncertain.

with some minor components (Figure 1a). They were resolved by isocratic chromatography of small aliquots (5 mL) on a large Cm-Sepharose column (Figure 1c). Both of these lectins are distinct from all rat S-Lac lectins described previously. Although the subunit molecular weight of A1, 14000, is similar to that of rat lung lectin RL-14.5, the isoelectric point of A1, pI 6.3, is quite different, and A1 reacts only weakly with an antiserum raised against RL-14.5 (Table I). It also differs from RL-14.5 in behaving as a monomer, whereas RL-14.5 behaves as a dimer on gel filtration. Unlike the rat lectins studied here and previously, RI-A1 has very little alanine (Table II). Fraction RI-A1 contains minor components with pI's of 5.8 and 5.9.

Fraction A2 contains a major component with a subunit molecular weight of 19000 and a minor component with a subunit molecular weight of 20000. The major component with an isoelectric point of 6.1 was distinct from minor components (together about 10%) with isoelectric points of 5.7, 6.8, and 6.9. This major component, designated RI-A2, behaved as a monomer on gel filtration and did not react significantly with antisera raised against RL-14.5, RL-18, and RL-29.

Rat Intestinal Lectin B. The major component of fraction B is similar to the rat lung lectin RL-18 (Cerra et al., 1985). It bound to DEAE-Sepharose and was eluted with 100–125 mM NaCl under the conditions described in Figure 1a and by 50 mM NaCl under the conditions described in Figure 2a. The subunit molecular weight of 18000 and the isoelectric point, 5.1, are the same as RL-18. Both RI-B and RL-18 contain minor components with pI's of 4.9 and 5.4, and in addition, RI-B has other components with pI's between 5.3 and 6.0. Like RL-18, RI-B is monomeric and reacts strongly with anti-RL-18. When analyzed by N-terminal amino acid sequencing, RI-B gave a mixture of residues in each cycle, and a major sequence could not be clearly identified. In contrast, the N-terminus of RL-18 was blocked.

Rat Intestinal Lectin C. Rat intestinal lectin C is probably identical with the rat lung lectin RL-14.5. It bound to DEAE-Sepharose and was eluted with 200 mM NaCl. The subunit molecular weight was 14500, and it was dimeric in its native form. Isoelectric focusing showed several closely spaced bands, similar to RL-14.5, between pI 4.3 and 4.9, the major being at pI 4.7. RI-C had the same reactivity with anti-RL-14.5 as authentic RL-14.5.

Rat Intestinal Lectins D and E. RI-D and RI-E were obtained in smaller amounts and lesser purity than the others and therefore were characterized in less detail. On the basis of the properties we examined, they appear to be distinct from the other rat intestinal lectins as well as other known rat S-Lac lectins. RI-D was obtained in the flow-through fraction of the Cm-Sepharose column, although slightly retarded. It was enriched in the lectin mixture bound to an overloaded Lac–Sepharose column (Figure 2b). Its subunit molecular weight is 19000, and it behaves as a monomer on gel filtration. It ran off the basic side of the isoelectric focusing gel in a gradient of pH 3–9. If the electrophoresis was not run to equilibrium, two major and two minor bands running slightly more to the basic side than α- chymotrypsin (pI 8.8) were observed. Fraction RI-D showed a distinct but weak reaction with an

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MI-C                        GvPaGPLTVPYDlPLPGGVMPR
CBP-35      111 sGgYPAtGPyGvPaGPLTVPYDlPLPGGVMPRMLITIMGT 150
IgEBP       110 PGAYPATGPFGAPTGPLTVPYDMPLPGGVMPRMLITIIGT 149
RI-F                        GAPTGPLTVPYDMPLPGGVMPRMLIT
RI-G                     maGpPifnppVPYvgtLqGGl
RI-H                  mlpvmaGpPifnppVPYvgtLqGGltaRrt
RI-H                        GpPifnppVPYvgtLqGGltaRrxI
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FIGURE 4: N-Terminal amino acid sequences of some intestinal lectins. Parts of the published sequences of rat IgE-binding protein (Albrandt et al., 1987) (that based on our unpublished data appears to be identical with RL-29[2]) and the mouse lectin CBP-35 (Jia & Wang, 1988) are compared with the N-terminal amino acid sequences obtained in this study. The sequence of IgEBP was used as the basis for alignment. Identical residues are shown by capital letters and nonidentical residues with lower case letters. For fraction RI-H, the two sequences detected are both shown (see text).

antiserum specific for RL-18.

RI-E bound weakly to Cm-Sepharose, was eluted with about 10–20 mM NaCl, and was recovered in fractions collected at the up slope of the peak corresponding to RI-F. Its subunit molecular weight is about 17 000, and the native protein behaves as a monomer. Isoelectric focusing showed a main component with p*I* 6.8, but other more basic components were also present. It showed some reactivity with an antiserum specific for RL-29. This may be due to a contaminating component of RI-F in this fraction.

Rat Intestinal Lectin F. RI-F bound to Cm-Sepharose and was eluted with about 40 mM NaCl under one set of conditions (Figure 2b) or with about 150 mM NaCl under another set of conditions (Figure 1b). Its subunit molecular weight is 17 500, and it behaves as a monomer on gel filtration. In isoelectric focusing studies, it ran off the gel at the basic side. Under nonequilibrium conditions, it showed a major band running between RI-D and cytochrome *c* (p*I* 9.6) and in addition, a slightly less basic band.

Despite these properties, which distinguish it from other known rat S-Lac lectins, RI-F was found to be immunologically related to RL-29, since it reacted strongly with an antiserum specific for that lectin but not with antisera raised against RL-14.5 or RL-18. This relationship was also indicated by the identity of the 26 N-terminal amino acids of RI-F (Figure 4) with residues 120–145 of a rat IgE-binding protein (Albrandt et al., 1987), that is related (Laing et al., 1989) to or identical with RL-29.[2] RI-F is also related to the mouse lectin CBP-35, since, of the N-terminal 26 amino acids of RI-F, 23 are identical with those found at positions 121–146 of CBP-35 (Figure 4).

These findings raised the possibility that RI-F is the product of proteolytic degradation of an intestinal form of RL-29. For this reason, it was of interest to determine if any intact RL-29 was present in the total S-Lac lectin preparation from intestine. Immunoblots of the total intestinal lectin mixture showed intense staining at M_r 17 000 due to RI-F, but no staining at M_r 29 000. In order to optimize the detection of small amounts of RL-29, a mixture of intestinal S-Lac lectins was applied to a DEAE column, and the flow-through fraction, which is enriched in RL-29 (Cerra et al., 1985), was examined by immunoblotting (Figure 5). Reaction of the blot with RL-29 produced intense staining at about M_r 17 000, corresponding to RI-F. This is seen as a broad band since the blot was heavily overloaded with this lectin. In contrast, no staining at M_r 29 000 was observed.

[2] RL-29 has been partially sequenced by mass spectrometry and tandem mass spectrometry of tryptic peptides. The sequence obtained so far (about 60%) is identical with the sequence deduced from a cDNA sequence of an IgE binding protein from rat (Albrandt et al., 1987; L. Poulter, H. Leffler, A. Burlingame, and S. Barondes, unpublished results).

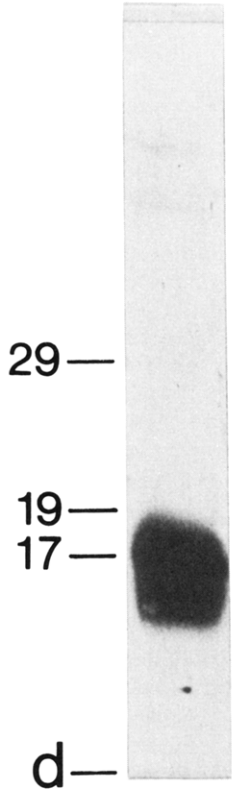

FIGURE 5: Western blot of a rat intestinal lectin fraction stained with an antiserum specific for RL-29 (Cerra et al., 1985). The fraction is the flow-through from a DEAE column. It contained RI-A1, RI-A2, and RI-D to H and should contain RL-29, if it were present (Cerra et al., 1985). The numbers are the relative molecular weights $\times 10^{-3}$ of RL-29, RI-A2, and RI-H used as standards, and (d) shows the mobility of the dye front.

Rat Intestinal Lectins G and H. Lectins G and H both bound to Cm-Sepharose and were eluted with 200 and 300 mM NaCl, respectively, under the conditions used in Figure 1b, or with 80 and 100 mM NaCl, respectively, under the conditions used in Figure 2b. Both had apparent subunit molecular weights of about 17 000, and both were monomeric in their native form. Both ran off the gel on the basic side on isoelectric focusing between pH 3 and 9. Under nonequilibrium conditions, RI-H showed one major band running at the same rate as the major band of RI-F and another running slightly faster. RI-G ran slightly slower than the major band of RI-H.

RI-G and RI-H were shown to be closely related on the basis of N-terminal amino acid sequencing. The H fraction shown in Figure 2b gave a major sequence of 30 residues starting with Met (Figure 4). This sequence accounted for 70% of the yield in each cycle. In addition, 20% of the yield in each cycle gave a minor sequence, starting with the Gly in position 7 of the major sequence. In another sequencing experiment using the H fraction shown in Figure 1b, the sequence starting with Gly was the major sequence, accounting for 60% of the yield in each cycle, while the sequence starting with Met accounted for 30%. Thus, fraction H contains at least two components which differ in that six additional N-terminal amino acids are present in one and not the other. Fraction G gave a heterogeneous N-terminal sequence, but major signals (over 50% of the yield) were clearly identified in each cycle. This sequence was identical with residues 5–25 of one of the fraction H sequences (Figure 4). Thus, RI-G may be a shorter form of RI-H.

The sequences of RI-G and RI-H showed some homology to RI-F, but marked differences were predominant (Figure

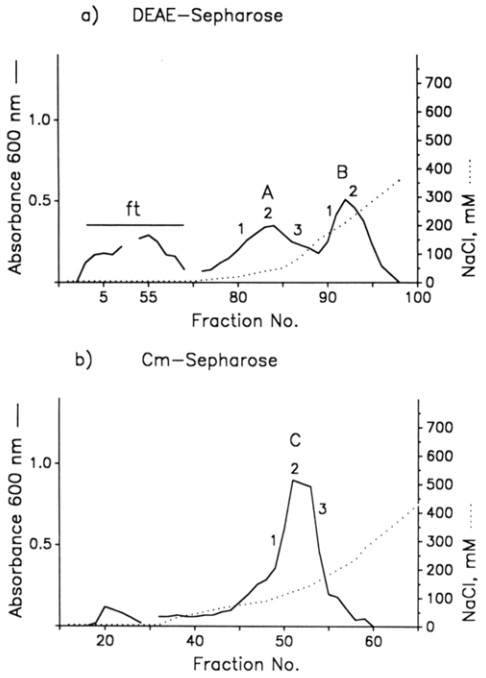

FIGURE 6: Ion-exchange chromatography of mouse intestinal lectins. The lectin mixture derived from the extraction of 150 g of mouse intestines and affinity chromatography on a 300-mL Lac–Sepharose column was used. The lectins were fractionated on (a) DEAE–Sepharose followed by (b) Cm-Sepharose as described in Figure 1a,b. Peaks are marked with letters, and single fractions used for further study are marked with numbers (see Figure 7).

4). RI-G and RI-H did not react with anti-RL-29, anti-RL-14.5, or anti-RL-18. The results indicate that both RI-G and RI-H are new members of the family of S-Lac lectins.

Mouse Intestinal Lectins. Because of known structural differences in β-galactoside-containing glycoconjugates of rat and mouse intestine, we compared the pattern of intestinal S-Lac lectins in those species. Mouse intestinal S-Lac lectins were isolated by using Lac–Sepharose and fractionated by ion-exchange chromatography (Figure 6) under conditions identical with those used for the rat intestine (Figure 1). As with rat, mouse intestine contains S-Lac lectins (Figure 7). In a few cases, the related mouse and rat lectins were apparent, but the relationships of others will require further study.

Fraction MI-A contained two components with subunit molecular weights 19000 and 20000. On IEF, we found major components at p*I* 6.1 and 5.4 and minor ones at p*I* 5.7 and 6.7. Therefore, MI-A is most similar to RI-A2. MI-A also bound and eluted from DEAE-Sepharose at a similar salt concentration as RI-A2. MI-A did not react with any of the antisera raised against the rat lung lectins. MI-B contained a component with subunit molecular weight of 14500 that reacted with anti-RL-14.5 and had similar chromatographic properties and only slightly different isoelectric points when compared with RL-14.5 or RI-C. It is probably the mouse form of RL-14.5 and RI-C.

Fraction C from Cm-Sepharose chromatography of the mouse lectins contained one major and several minor components. The major one had a subunit molecular weight of 17000 and isoelectric point >9. Under nonequilibrium conditions, it ran close to RI-F. Minor components with subunit molecular weights of 19000 and 20000 and p*I*'s of 6.7 and >9 were also observed. Under nonequilibrium conditions, the latter ran close to RI-D. On dot immunoblots, fraction C reacted with anti-RL-29 but less strongly than RI-F.

When mouse fraction C was analyzed by Edman degradation, a clear major N-terminal sequence of 22 amino acids was

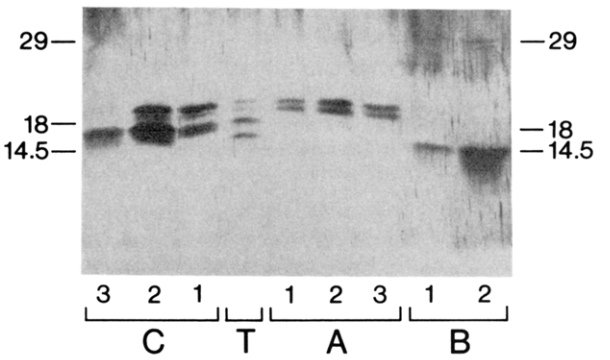

FIGURE 7: SDS–PAGE of mouse intestinal lectin fractions. The designations A–C and numbers refer to fractions isolated by ion-exchange chromatography as described in Figure 6. T is the Lac–Sepharose eluate. Aliquots of 1/300 of the fractions containing between 100 and 1000 ng of protein were applied to the gel. The mobility of RL-14.5, RL-18, and RL-29, rat lung lectins of known molecular weight, is shown at the margins.

obtained. This was identical with residues 121–142 of CBP-35 and closely resembled the N-terminal sequence of RI-F (Figure 4). On the basis of the recoveries we obtained, it is likely that this sequence derives from the major component of fraction C. A minor sequence, which was not homologous to any of the other sequences, was also observed (not shown). The sequence data suggest that the major component of MI-C is the mouse analogue of RI-F and that it may be identical with the C-terminal carbohydrate-binding part of CBP-35.

We found no mouse lectin eluted from the Cm-Sepharose after MI-C, where RI-G and RI-H would be eluted. We found no mouse lectin which, like RI-B, had an apparent subunit molecular weight of 18000.

Carbohydrate-Binding Specificity of RI-H and MI-C. To relate the specificity of the major rat and mouse intestinal lectins to those examined previously, we studied the inhibitory effects of a panel of oligosaccharides on their binding to Lac–Sepharose. The specific binding of radiolabeled RI-H or MI-C to small volumes of Lac–Sepharose beads was about 20% under the conditions used. Pelleting of lectin in the absence of beads or in the presence of underivatized Sepharose beads was about 5% for RI-H and 2% for MI-C, and the results were corrected for this. The interaction of saccharides with the lectins was assessed by determining their relative potency as inhibitors of Lac–Sepharose binding (Table III). The concentration of lactose giving 50% inhibition was 1.2 mM for RI-H and 0.5 mM for MI-C. The results with the panel of saccharides indicate that MI-C and RI-H bind to the same general structural determinants of lactose as the other lectins studied previously (Leffler & Barondes, 1986; Sparrow et al., 1987). Thus, the compounds in which these determinants were free (2, 4, 5, 9, and 10) all bound as well as or better than lactose while the compounds in which the determinants were blocked (3, 6, and 7) all had significantly lower potency. Compound 4 showed the highest activity for both lectins, indicating the resemblance of RI-H and MI-C to RL-29 (Leffler & Barondes, 1986) as opposed to RL-14.5 or RL-18. Differences in potencies of some saccharides (e.g., compounds 8, 11, and 12) in inhibiting binding to RI-H compared to MI-C may indicate differential specificity toward larger saccharides.

Discussion

This paper shows that rat intestine is especially rich in S-Lac lectins, which comprise about 1% of the total soluble proteins of this tissue. Of these, RI-B and RI-C appears to be identical with or closely related to rat S-Lac lectins described previously.

Table III: Inhibition of Binding of the Major Rat and Mouse Intestinal Lectins to Lac–Sepharose by a Series of Saccharides, Expressed as Inhibitory Activity of Each Compound Relative to Lactose

Compd	Formula	Relative activity[a]	
		RI-H	MI-C
1.	Galβ1-4Glc	1	1
2.	Galβ1-4Glc (2 Fucα1)	1.1	1
3.	Galβ1-4Glc (3 Fucα1)	<0.05	<0.03
4.	GalNAcα1-3Galβ1-4Glc (2 Fucα1)	10	16
5.	NeuAcα2-3Galβ1-4Glc	n.t.	0.5
6.	NeuAcα2-6Galβ1-4Glc	n.t.	0.07
7.	Galα1-4Galβ1-4Glcβ-O-Eth	n.t.	<0.07
8.	GalNAcβ1-3Galα1-4Galβ1-4Glc	<0.05	0.5
9.	Galβ1-4GlcNAc	0.9	3.4
10.	Galβ1-3GlcNAc	0.4	0.8
11.	Galβ1-3GalNAc	0.2	0.5
12.	GalNAcβ1-3Galα-O-Me	0.2	0.9

[a] The inhibitory activity of a series of concentrations of each substance was tested in the Lac–Sepharose binding assay. The relative activity is the concentration (M) of lactose giving 50% inhibition divided by the concentration (M) of the test saccharide giving 50% inhibition. [b] nt means not tested.

However, others are new, including RI-H, the predominant rat intestinal lectin.

A particularly interesting rat lectin clearly related to those previously identified is RI-F, whose first 26 amino acids, determined by N-terminal sequencing, are identical with residues 120–145 of a rat carbohydrate-binding protein (Laing et al., 1989) which we have reason to believe is identical with RL-29.[2] Furthermore, the first 22 amino acids of its mouse homologue, MI-C, are identical with residues 121–142 of the mouse lectin CBP-35. Since Jia and Wang (1988) presented evidence that the C-terminal part of CBP-35 is the carbohydrate-binding domain and that the N-terminal domain has another function, RI-F and MI-C may represent alternative functional forms containing only the carbohydrate-binding domains. Whether they are generated in vivo or are in vitro proteolytic artifacts has not yet been established conclusively.

The six other rat lectins identified here all appear different from lectins previously isolated from this species. The most abundant, RI-H, is the major rat intestinal lectin. It was isolated in two forms. One begins with a methionine which could be the starting residue of the translated protein, whereas the shorter form might have arisen by specific proteolytic cleavage. The lectin RI-G is clearly related to RI-H, beginning four amino acid residues downstream from what appears to be the start of the longer form of RI-H. RI-G also begins with a methionine and could be the product of an alternative start site. Partial sequences of RI-A1, RI-A2, RI-D, and RI-E have not yet been obtained, because they are either blocked or relatively scarce.

The mouse intestinal lectins we isolated were quite similar to corresponding rat lectins. The most notable difference is that mouse intestine does not contain an obvious lectin with physiochemical properties of RI-H, the major intestinal lectin of rat. However, MI-C, the major intestinal lectin of mouse, has similar binding properties.

The results described here have bearing on ongoing attempts to classify vertebrate lectins. Drickamer (1988) recently suggested that they could be grouped together as "C-type" or "S-type". The former were united by a dependence on calcium ions for activity (hence C-type) and by structural homologies, although they bound to different sugars. The latter consisted largely of variants, from different species, of a soluble lactose-binding lectin with subunit molecular weights of about 14000–16000. Since this lectin requires reducing conditions to maintain activity, the group was tentatively designated "S-type" (for sulfhydryl) despite the fact that one member, which requires reducing conditions, is known to not have a sulfhydryl group (Levi & Teichberg, 1981). The other lectin grouped with these was CBP-35 which shows clear structural homology (Jia & Wang, 1988). Although it is active in the presence of thiol reagents, indicating that it does not depend on disulfide bonds, it may or may not be dependent on reduced sulfhydryl groups for activity. This is also the case with the new lectins described here.

The present results introduce structural data on other members of this group. When coupled with prior work, they suggest an alternative way of naming these lectins. One defining characteristic is that all are soluble without detergents, a property that is undoubtedly biologically significant. Furthermore, all known members are united by a fundamental similarity in their carbohydrate-binding site. Of the rat (Leffler & Barondes, 1986) and human (Sparrow et al., 1987) lectins of this group that were studied in detail, all interact with lactose not only by binding to its galactose residue but also by binding to components of its glucose residue, and all appear to bind to the same face of the lactose molecule (Sparrow et al., 1987; Leffler & Barondes, 1986). Since galactose alone, or combined with other saccharides, is a much poorer ligand than lactose, the term galaptin (Harrison & Chesterton, 1980) proposed to designate some of these lectins does not adequately reflect their specificity.

As shown here and in previous work, this family also appears to be united by structural homology. This was first suggested from the structure of a cDNA clone from chicken skin (Ohyama et al., 1986) and of two cDNA clones isolated from a human hepatoma cDNA library (Gitt & Barondes, 1986) and then extended by the sequencing of a cDNA encoding CBP-35 (Jia & Wang, 1988). The present studies indicate that structural homology extends to RI-F, RI-G, RI-H, and MI-C, the only new lectins whose N-terminal sequence we determined. Furthermore, unpublished work indicates that the sequence of a tryptic peptide from RL-18 shares 9 of its 12 amino acids with a related region of RL-29.[3] It also seems likely that the elastin receptor, a soluble lactose-binding protein, shares some amino acid sequence with RL-14.5 since these proteins are immunologically cross-reactive (Hinek et al., 1988).

Given all these relationships, we suggest that the lectins described here and in previous studies be recognized as members of a specific family. At present, defining properties include their solubility in the absence of detergents and their affinity for lactose. Because of these properties, S-Lac lectins (for soluble lactose-binding vertebrate lectins) seem to be a reasonable designation.

ACKNOWLEDGMENTS

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[3] S. Massa, H. Leffler, and S. Barondes, unpublished results.

REFERENCES

Albrandt, K., Orida, N. K., & Liu, F.-T. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6859–6863.

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

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

Barondes, S. H. (1986) in *The Lectins* (Liener, I. E., Sharon, N., & Goldstein, I. J., Eds.) pp 437–466, Academic Press, Orlando, FL.

Barondes, S. H. (1988) *Trends Biochem. Sci. (Pers. Ed.) 13*, 480–482.

Bidlingmeyer, B. A., Cohen, S. A., & Tarvin, T. L. (1984) *J. Chromatogr. 336*, 93–104.

Bjork, S., Breimer, M. E., Hansson, G. C., Karlsson, K.-A., & Leffler, H. (1987) *J. Biol. Chem. 262*, 6758–6765.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Breimer, M. E., Hansson, G. C., Karlsson, K.-A., & Leffler, H. (1982) *J. Biol. Chem. 257*, 557–568.

Carding, S., Childs, R. A., Thorpe, R., Spitz, M., & Feizi, T. (1985) *Biochem. J. 228*, 147–153.

Carlstedt, I., Sheehan, J. K., Corfield, A. P., & Gallagher, J. T. (1985) *Essays Biochem. 20*, 40–76.

Cerra, R. F., Gitt, M. A., & Barondes, S. H. (1985) *J. Biol. Chem. 260*, 10474–10477.

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

Crittenden, S. L., Roff, C. F., & Wang, J. L. (1984) *Mol. Cell. Biol. 4*, 1252–1259.

Drickamer, K. (1988) *J. Biol. Chem. 263*, 9557–9560.

Friedman, M., Krull, L. G., & Cavine, J. F. (1970) *J. Biol. Chem. 245*, 3868–3871.

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

Hansson, G. C., Karlsson, K.-A., Leffler, H., & Stromberg, N. (1982) *FEBS Lett. 139*, 291–294.

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

Hinek, A., Wrenn, D. S., Mecham, R. P., & Barondes, S. H. (1988) *Science 239*, 1539–1541.

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

Imamura, T., Toyoshima, S., & Osawa, T. (1984) *Biochim. Biophys. Acta 805*, 235–244.

Jia, S., & Wang, J. L. (1988) *J. Biol. Chem. 263*, 6009–6011.

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

Laing, G. L., & Wang, J. L. (1988) *Biochemistry 27*, 5329–5334.

Laing, G. L., Robertson, M. W., Gritzmacher, C. A., Wang, J. L., & Liu, F. T. (1989) *J. Biol. Chem. 264 (4)*, 1907–1910.

Leffler, H., & Barondes, S. H. (1986) *J. Biol. Chem. 261*, 10119–10126.

Levi, G., & Teichberg, V. I. (1981) *J. Biol. Chem. 256*, 5735–5740.

Mbamalu, G. M., & Zalik, S. E. (1987) *Wilhelm Roux's Arch. Dev. Biol. 196*, 176–184.

Morrisey, J. H. (1981) *Anal. Biochem. 117*, 307–310.

Moutsatsos, I. K., Wade, M., Schindler, M., & Wang, J. L (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6452–6456.

Ohara, T., & Yamagata, T. (1986) *Biochim. Biophys. Acta 884*, 344–354.

Ohyama, Y., Hirabayashi, J., Oda, Y., Ohno, S., Kawasaki, H., Suzuki, K., & Kasai, K. (1986) *Biochem. Biophys. Res. Commun. 134*, 51–56.

Porath, J., & Ersson, B. (1975) in *Proceedings of the Symposium on New Approaches for inducing Natural Immunity to Pyrogenic Organisms, Winter Park, Florida* (Robbins, J. B., Horton, R. E., & Krause, R. M., Eds.) pp 101–108, NIH, Bethesda, MD.

Roff, C. F., & Wang, J. L. (1983) *J. Biol. Chem. 258*, 10657–10663.

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

Towbin, H., Staehelin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.

Whitney, P., Maxwell, S., Ryan, U., & Massaro, D. (1985) *Am. J. Physiol. 248*, C258–C264.

Zalik, S. E., Milos, N., & Ledsham, I. (1983) *Cell Differ. 12*, 121–127.