Role of the Carboxyl-Terminal Lectin Domain in Self-Association of Galectin-3[†]

Ri-Yao Yang, Paul N. Hill, Daniel K. Hsu, and Fu-Tong Liu*

Division of Allergy, La Jolla Institute for Allergy and Immunology, 10355 Science Center Drive, San Diego, California 92121

Received June 12, 1997; Revised Manuscript Received January 15, 1998

ABSTRACT: Galectin-3 is a member of a large family of β-galactoside-binding animal lectins and is composed of a carboxyl-terminal lectin domain connected to an amino-terminal nonlectin part. Previous experimental results suggest that, when bound to multivalent glycoconjugates, galectin-3 self-associates through intermolecular interactions involving the amino-terminal domain. In this study, we obtained evidence suggesting that the protein self-associates in the absence of its saccharide ligands, in a manner that is dependent on the carboxyl-terminal domain. This mode of self-association is inhibitable by the lectin's saccharide ligands. Specifically, recombinant human galectin-3 was found to bind to galectin-3C (the carboxyl-terminal domain fragment) conjugated to Sepharose 4B and the binding was inhibitable by lactose. In addition, biotinylated galectin-3 bound to galectin-3 immobilized on plastic surfaces and the binding could also be inhibited by various saccharide ligands of the lectin. A mutant with a tryptophan to leucine replacement in the carboxyl-terminal domain, which exhibited diminished carbohydrate-binding activity, did not bind to galectin-3C−Sepharose 4B. Furthermore, galectin-3C formed covalent homodimers when it was treated with a chemical cross-linker and the dimer formation was completely inhibited by lactose. Therefore, galectin-3 can self-associate through intermolecular interactions involving both the amino- and the carboxyl-terminal domains and the relative contribution of each depends on whether the lectin is bound to its saccharide ligands.

Galectins are members of a growing family of β-galactoside-binding animal lectins. Presently, 10 members have been formally designated and more are likely to be identified (Barondes et al. 1994; Kasai & Hirabayashi, 1996; Gitt et al. 1995; Madsen et al. 1995; Hadari et al. 1995; Wada & Kanwar, 1997; Dyer et al. 1997). Galectin-3, the most characterized member of this family, contains from 243 to 286 amino acid residues (depending on species of origin) and is composed of a short amino-terminal stretch, a domain containing short tandem repeats composed primarily of tyrosine, proline, glycine, and alanine, and a carboxyl-terminal lectin domain (Albrandt et al. 1987; Cherayil et al. 1989, 1990; Jia & Wang, 1988; Raz et al. 1989, 1991; Robertson et al. 1990; Herrmann et al. 1993; Mehul et al. 1994). This lectin has been shown to have diverse biological activities in vitro, including activation of leukocytes (Frigeri et al. 1993; Zuberi et al. 1994; Liu et al. 1995; Truong et al. 1993; Yamaoka et al. 1995); modulation of cell adhesion (Kuwabara & Liu, 1996; Bao & Hughes, 1995); and induction of pre-mRNA splicing (Dagher et al. 1995). The associations between the expression of this lectin and cell growth, neoplastic transformation, and metastasis have been noted (Hamann et al. 1991; Voss et al. 1994; Raz et al. 1990) and our recent results suggest a role for galectin-3 in regulation of cell growth and apoptosis (Yang et al. 1996).

Many of the in vitro biological activities of galectin-3 have been attributed to the protein's multivalent binding of carbohydrate moieties (Frigeri et al. 1993; Yamaoka et al. 1995; Kuwabara & Liu, 1996), a property that was first suggested by its hemagglutination activity (Frigeri et al. 1990). Since galectin-3 possesses only one carbohydrate-binding site (Hsu et al. 1992), its multivalent behavior must be attributed to the formation of dimers or higher order multimers. In the absence of saccharide ligands, galectin-3 exists primarily as monomers in solution, as multimeric species have not been detected in gel-filtration experiments (Hsu et al. 1992; Massa et al. 1993). The observation of β-galactoside-inhibitable cooperative binding of galectin-3 to immobilized IgE (Hsu et al. 1992) and laminin (Massa et al. 1993) suggests the propensity of this lectin to self-associate under specific conditions. The amino-terminal region appears to be critical for the multivalent behavior of galectin-3, because the carboxyl-terminal domain fragment of galectin-3, while retaining affinity for β-galactoside ligands, lacks hemagglutination activity and does not exhibit similar cooperative binding (Hsu et al. 1992). The tendency of the amino-terminal regions to interact with each other is further suggested by the finding that the purified amino-terminal fragment efficiently self-associates (Mehul et al. 1994).

However, galectin-3 appears also to be able to self-associate in the absence of its saccharide ligands, as suggested by chemical cross-linking experiments (Hsu et al. 1992; Mehul et al. 1994) as well as the detection of dimers by gel electrophoresis and of structures suggestive of dimers by electron microscopy (Ochieng et al. 1993). Insight into the molecular basis for galectin-3's self-association was provided by our recent finding that galectin-3 has significant

[†] This is publication 158 of the La Jolla Institute for Allergy and Immunology. This work was supported in part by NIH Grants AI20958 and AI39620.

* Author to whom correspondence should be addressed.

S0006-2960(97)01409-8 CCC: $15.00
Published on Web 03/05/1998

sequence similarity with Bcl-2, a well-characterized repressor of apoptosis, and that these two proteins interact with each other (Yang et al. 1996). Bcl-2 family members are known to form homodimers and heterodimers (a property that is believed to be important for their apoptosis-regulating activities), in a fashion that is dependent on the highly conserved NWGR motif, which is also present in the lectin domain of galectin-3. Therefore, the carboxyl-terminal lectin domain of galectin-3 may also be involved in protein self-association. Moreover, the self-association mediated through the lectin domain may be disrupted by the occupancy of the carbohydrate-binding site, because peptide/peptide interaction between galectin-3 and Bcl-2 is inhibitable by lactose (Yang et al. 1996). We thus reevaluated galectin-3 self-association, by using recombinant galectin-3 carboxyl-terminal domain fragment (galectin-3C)[1] and a mutant lacking carbohydrate-binding activity and found that, in the absence of saccharide ligands, galectin-3 underwent self-association primarily through intermolecular interactions involving the carboxyl-terminal lectin domain.

EXPERIMENTAL PROCEDURES

Preparation of Recombinant Human Galectin-3 and Galectin-3C. Recombinant full-length human galectin-3 and its lectin domain [galectin-3C, residues 112−250 of galectin-3 (Robertson et al. 1990)] were produced in *Escherichia coli* and purified as described (Hsu et al. 1992; Liu et al. 1996).

Preparation of Biotinylated Human Galectin-3. Purified recombinant human galectin-3 (1 mg/mL in PBS/10% glycerol) was dialyzed against 0.1 M borate buffer, pH 8.5, and was then added to crystalline sulfo-NHS-biotin (Pierce, Rockford, MD) to achieve a reagent:protein molar ratio of 100: 1. The reaction mixture was incubated for 4 h at 4 °C and then dialyzed against PBS/10% glycerol.

Generation of a Galectin-3 Mutant with the Single Tryptophan^{181} in the Lectin Domain Replaced with Leucine. Human galectin-3 cDNA insert was excised from clone 2.2 (Robertson et al. 1990) with *Eco*RI and cloned into vector pBK-CMV (Stratagene, San Diego, CA), in the sense direction with respect to the CMV promoter. The resulting plasmid was used as a template for the W181L mutagenesis by overlapping PCR (Tsujimura et al. 1993). In the first round of PCR, primers 5′AATTAACCCTCACTAAAGGG3′ (T3 promoter sequence) and 5′TTCCCAAGTTATTATC-CGGCTTTG3′ were used to generate the upstream half of the cDNA encoding the W181L mutant. The downstream half of the mutated sequence was generated by using primers 5′TAACTTGGGAAGAAAGAC3′ and 5′GTAATACGACT-CACTATAGGGC3′ (T7 promoter sequence). The two PCR products were purified and then mixed with T3 and T7 primers in a third round of PCR to generate cDNA coding for galectin-3(W181L). The final PCR product was purified, digested with *Eco*RI, cloned back into pBK−CMV (pBK-CMV-W181L), and sequenced to confirm the mutation.

Expression of Galectin-3 and Galectin-3(W181L) in Jurkat Cells. The cDNAs for galectin-3 and galectin-3(W181L) were excised with *Eco*RI from clone 2.2 (Robertson et al. 1990) and pBK-CMV-W181L, respectively, and cloned into pEF1-neo, which was prepared by replacing the CMV promoter in the bicistronic vector pIRES1-neo (Clontech, Palo Alto, CA) with the human elongation factor 1α promoter (Uetsuki et al. 1989) that has been shown to be highly active in a variety of cell types (Mizushima & Nagata, 1990). Plasmid DNA was purified with Qiagen columns (Qiagen, San Diego, CA) and transfected into Jurkat cells by electroporation, as described previously (Yang et al. 1996).

Determination of Binding of Galectin-3 and Galectin-3(W181L) to Lactosyl-Sepharose 4B and Galectin-3C−Sepharose 4B. Purified recombinant human galectin-3C was coupled to CNBr-activated Sepharose 4B (Pharmacia, Piscataway, NJ) according to the manufacturer's procedure. The conjugates typically contained 1 mg of protein/mL of beads. Lactosyl- and sucrosyl-Sepharose 4B were prepared as described (Levi & Teichberg, 1981). Two hundred microliters of 20 nM recombinant human galectin-3 in binding buffer (20 mM HEPES, pH 7.5, 142.5 mM KCl, 5 mM $MgCl_2$, 0.2% NP-40) or cell lysates (10^7 cells/mL) [prepared as described (Liu & Orida, 1984)] were incubated with 10 μL of lactosyl-, sucrosyl-, or galectin-3C−Sepharose 4B at 4 °C for 1 h with constant mixing. After the beads were washed three times with 1 mL of binding buffer, bound proteins were eluted with 10 μL of SDS sample buffer and then analyzed by immunoblotting essentially as described (Liu et al. 1996). Briefly, proteins separated by SDS−PAGE were transferred to Immobilon membrane (Millipore, Bedford, MA) and detected with a mouse monoclonal anti-galectin-3 antibody, B2C10 (Liu et al. 1996). The immunoblots were developed with the ECL system (Amersham, Arlington Heights, IL). For demonstration of inhibition of binding by saccharides, the various proteins were incubated with the conjugated Sepharose 4B in the presence of 25 mM lactose or sucrose.

Assay for Binding of Biotinylated Galectin-3 to Galectin-3 Immobilized on Plates. Immulon-4 plates (96-well) were coated with 10 μg/mL human galectin-3 in PBS overnight at 4 °C and blocked with 1% BSA (Pentex fraction V) in PBS for 1 h at room temperature. One hundred microliters of biotinylated galectin-3 (40 nM) in 1% BSA/PBS/0.05% Tween (PBSA/Tween) containing serial dilutions of various saccharides was added to each well and the plates were incubated for 4 h at 4 °C. The plates were washed with PBS and incubated with avidin−horseradish peroxidase conjugate (Bio-Rad, Hercules, CA), diluted in PBSA/Tween, and developed with ABTS [2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)]. Absorbance at 405 nm was recorded using a Titertek Multiskan 96-well spectrophotometer.

Assay for Binding of Biotinylated Galectin-3 to IgE-Coated Plates. The procedure was essentially identical to that described for binding of biotinylated galectin to galectin-3-coated plates, except the plates were coated with 10 μg/mL mouse anti-DNP IgE (Liu et al. 1980).

Reaction of Galectin-3 and Galectin-3C with Chemical Cross-Linker. Protein self-association was demonstrated by chemical cross-linking essentially as described (Patel et al. 1995) except that dimethyl pimelimidate (DMP, Pierce, Rockford, IL), which has a shorter chain length than dimethyl suberimidate, was used as the cross-linker. Briefly, 8 μg of recombinant galectin-3 or 4 μg of recombinant galectin-3C

[1] Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); BSA, bovine serum albumin; DMP, dimethyl pimelimidate; galectin-3C, carboxyl-terminal domain of galectin-3; PBS, phosphate-buffered saline, pH 7.2.

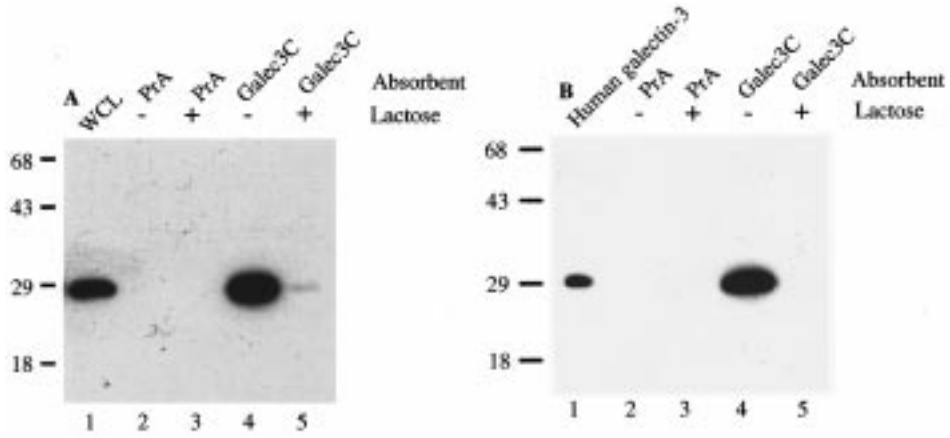

FIGURE 1: Binding of galectin-3 to immobilized galectin-3C and inhibition by lactose. (A) An aliquot of lysate (200 μL) from HeLa cells was mixed with protein A−Sepharose 4B (lanes 2 and 3) or galectin-3C−Sepharose 4B (lanes 4 and 5) in the absence (lanes 2 and 4) or presence (lanes 3 and 5) of lactose. The bound protein was eluted and analyzed by immunoblotting. Lane 1 represents the unabsorbed whole cell lysate (WCL; 20 μL). (B) Purified recombinant galectin-3 was incubated with protein A−Sepharose 4B (lanes 2 and 3) or galectin-3C−Sepharose 4B (lanes 4 and 5) in the absence (lanes 2 and 4) or presence (lanes 3 and 5) of lactose. The bound proteins were eluted and analyzed by immunoblotting. The numbers on the left margin denote molecular weight ($\times 10^{-3}$) markers. PrA = protein A; galec3C = galectin-3C.

was incubated with 1 mM DMP in 0.1 mL of buffer (10 mM HEPES, pH 8.0, and 0.1 M NaCl), in the presence or absence of 25 mM lactose, for 10 or 30 min at 25 °C. Reaction was stopped by adding 1 M glycine to a final concentration of 0.1 M. Protein was then denatured by boiling for 2 min in SDS sample buffer and separated by SDS−PAGE on a 12.5% polyacrylamide gel followed by immunoblotting with a mouse monoclonal anti-galectin-3 antibody, B2C10 (Liu et al. 1996), for detection of galectin-3, or with rabbit polyclonal anti-galectin-3 antibodies (Liu et al. 1995), for detection of galectin-3C. Recombinant galectin-3 affinity-purified by using lactosyl-Sepharose 4B invariably contains a small amount of degradation products with a truncated amino-terminal region (Hsu et al. 1992). Therefore, in the immunoblotting analysis, we used the monoclonal antibody B2C10, which recognizes an epitope near the amino-terminus of galectin-3, to avoid detection of cross-linked species corresponding to the degradation products. However, we used polyclonal antibodies, which bind to the carboxyl-terminal domain as well as the amino-terminal region, for detection of cross-linked products of galectin-3C.

RESULTS

Galectin-3 Binds to Galectin-3C−Sepharose 4B in a Lactose-Inhibitable Manner. A lysate of HeLa cells was incubated with protein A−Sepharose 4B or galectin-3C−Sepharose 4B in the presence or absence of lactose. The bound protein was eluted and analyzed by immunoblotting. As shown in Figure 1A, galectin-3 present in the lysate bound to galectin-3C−Sepharose 4B but not to protein A−Sepharose 4B (lane 4 vs lane 2). The binding was nearly completely inhibitable by lactose (lane 5). By densitometric analysis of chemiluminescence exposure on film (comparing lanes 1 and 4), it was determined that approximately 13% of galectin-3 in the original lysate bound to galectin-3C−Sepharose 4B. The fact that galectin-3 contained in cell lysates bound to galectin-3C−Sepharose 4B suggests that, even in the presence of a large excess of other proteins, galectin-3 can specifically self-associate.

To confirm the above finding, we tested the binding of purified recombinant human galectin-3 to galectin-3C−Sepharose. Again, galectin-3 bound to galectin-3C−Sepharose 4B and not to protein A−Sepharose 4B (Figure 1B, lane 4 vs lane 2) and the binding was completely inhibitable by lactose (lane 5). By densitometric analysis, it was determined that approximately 23% of galectin-3 in the initial mixture bound to galectin-3C−Sepharose 4B.

Comparison of Saccharide Inhibition of Galectin-3 Binding to Galectin-3 and to IgE. The above results suggested that galectin-3 interacts with galectin-3 in a manner that is similar to its binding to glycoconjugates, such as IgE. We thus studied the inhibition of these two interactions by various saccharides. As shown in Figure 2, the relative ability of various saccharides in inhibiting galectin-3−galectin-3 interaction (panel A) parallels the inhibition of galectin-3−IgE interaction (panel B). IgE at lower concentrations (<1 μM) promoted the binding of galectin-3 to galectin-3-coated plate. This is most likely due to bridging of biotinylated galectin-3 to immobilized galectin-3 by IgE, which carries several β-galactoside-containing oligosaccharide chains that the lectin can bind to (Baenziger et al. 1974). At higher concentrations of IgE, this bridging effect cannot take place and IgE then inhibits the galectin-3/galectin-3 interaction similarly to other galactosides.

A Single Tryptophan to Leucine Mutation in the Lectin Domain of Galectin-3 Results in a Concomitant Diminution of Carbohydrate-Binding Activity and Lectin Domain-Mediated Self-Association. The single tryptophan residue in the carbohydrate-binding domain of galectin-3 has been shown to be in direct contact with galactose at the carbohydrate-binding site of galectin-3 (Hakon Leffler, University of California, San Francisco, personal communication). The corresponding residue in galectin-2 is also involved in carbohydrate-binding (Liao et al. 1994; Lobsanov et al. 1993). The NWGR motif in Bcl-2 is directly involved in Bcl-2/Bcl-2 homodimerization and Bcl-2/Bax heterodimerization (Hanada et al. 1995). Therefore, the notion that the tryptophan residue forms a part of the galectin-3/galectin-3 self-association contact site is attractive. A galectin-3 mutant

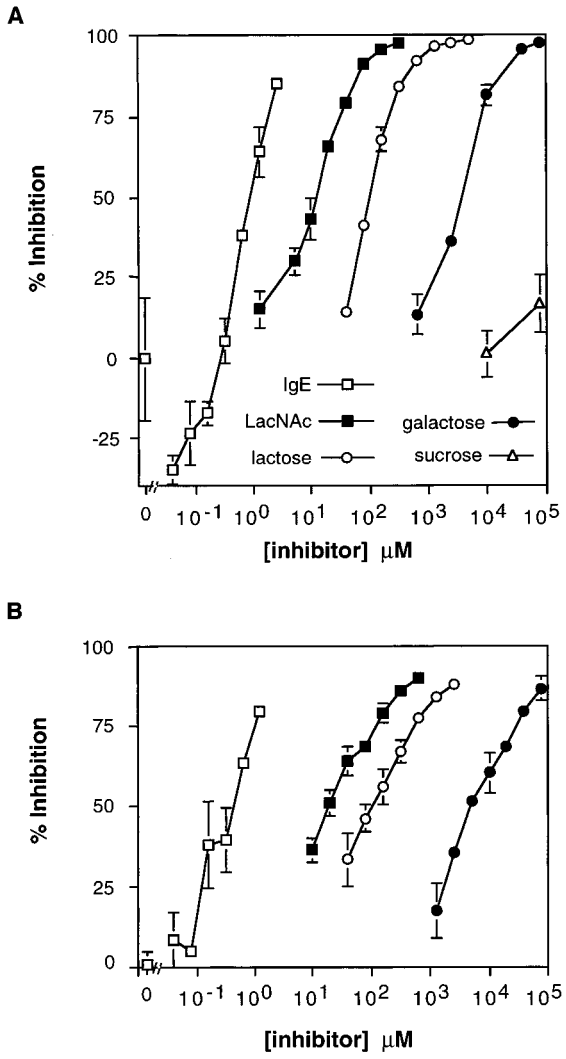

FIGURE 2: Inhibition by saccharides of binding of biotinylated galectin-3 to galectin-3-coated and IgE-coated plates. Biotinylated galectin-3 was added to microtiter wells coated with galectin-3 (panel A) or IgE (panel B) in the presence of serial dilutions of various saccharides and IgE. The bound biotinylated protein was detected as described in Experimental Procedures. Data are presented as mean ± SD of triplicate determinations. Similar results were obtained in three separate experiments. LacNac = *N*-acetyllactosamine.

with this residue replaced with leucine was generated and tested first for its ability to bind lactose. As shown in Figure 3A, while, as expected, galectin-3 bound to lactosyl-Sepharose (lane 3) and not to sucrosyl-Sepharose 4B (lane 4), galectin-3(W181L) failed to bind to either adsorbent (lanes 5 and 6). The ability of galectin-3(W181L) to bind to galectin-3C−Sepharose 4B was then tested. As shown in Figure 3B, in significant contrast to galectin-3, which bound to galectin-3C (lane 2), the mutant did not exhibit any binding (lane 4). We also found that while GST fusion protein of galectin-3C bound to galectin-3C−Sepharose, GST fusion protein of galectin-3C(W181L) failed to bind. In contrast, GST-galectin-3(W181L) as well as GST-galectin-3 bound to galectin-3-Sepharose (data not shown). These results suggest that the amino-terminal region of galectin-3(W181L) retains the ability to interact with the amino-terminal region of the wild-type galectin-3.

Demonstration of Lactose-Inhibitable Self-Association of Galectin-3 by Chemical Cross-Linking. The ability of galectin-3 to self-associate in a lactose-inhibitable manner was examined further by chemical cross-linking. Purified recombinant human galectin-3 was treated with dimethyl pimelimidate (DMP) in the presence or absence of lactose and the reaction mixture was then analyzed by immunoblotting. A molecular species with M_r of approximately 50 000, corresponding to that of galectin-3 dimer, was clearly detected after 10 min of reaction and the amount of this product was further increased after 30 min (Figure 4A). Significantly lower amounts of galectin-3 dimer were formed in the presence of lactose (lane 3 vs lane 2 and lane 5 vs lane 4). By densitometry, it was determined that the quantity of galectin-3 dimer formed in the presence of lactose was 45% and 30% of that formed in the absence of lactose, at 10 and 30 min, respectively. To demonstrate the role of galectin-3C in self-association, the chemical cross-linking experiment was performed similarly with galectin-3C. As shown in Figure 4B, a dimer of galectin-3C was clearly detectable after galectin-3C was treated with DMP for 30 min. Importantly, the dimer formation was completely inhibited in the presence of lactose (lane 5 vs lane 4).

DISCUSSION

Previously, on the basis of galectin-3's hemagglutination activity and cooperative binding to immobilized IgE and the fact that its carboxyl-terminal lectin domain fragment lacks these activities, we proposed that upon binding to multivalent saccharide ligands, galectin-3 self-associates in a manner that involves intermolecular interactions of the amino-terminal domain (Figure 5, model d). The major conclusion of the present study is that galectin-3 molecules can also interact with each other in a manner that is dependent on its carboxyl-terminal lectin domain and this interaction is inhibitable by saccharide ligands of the lectin. This was demonstrated by the binding of galectin-3 to galectin-3C immobilized on Sepharose 4B beads and the binding of biotinylated galectin-3 to galectin-3 immobilized on plastic plates. Additional support was provided by the formation, in a lactose-inhibitable fashion, of stable homodimers when galectin-3 or galectin-3C was treated with a chemical cross-linker. The involvement of the carboxyl-terminal domain is further highlighted by the observation that mutation of a tryptophan residue in this domain (W181L) results in a loss of both carbohydrate-binding and self-association activities.

The formation of homodimers of galectin-3 and galectin-3C induced by chemical cross-linking confirmed previous chemical cross-linking studies by this and another group (Hsu et al. 1992; Mehul et al. 1994). Consistent with these earlier studies, the cross-linking was relatively inefficient. However, while trimers and higher order oligomers were detected in these earlier studies, only dimers were noted in the present study, which is probably due to the use of different chemical cross-linkers. In particular, in our earlier study, 1,5-difluoro-2,4-dinitrobenzene was used, which, unlike DMP, reacts with thiol, imidazole, and phenolic side chains in addition to amino groups, and tyrosine is a major residue present in the amino-terminal region of galectin-3. In addition, in our earlier studies (Hsu et al. 1992) radioiodinated galectin-3 was used to facilitate the detection of the cross-linked products. Nevertheless, the most significant new finding made in the present study is that the stable homodimer formation is inhibitable by lactose.

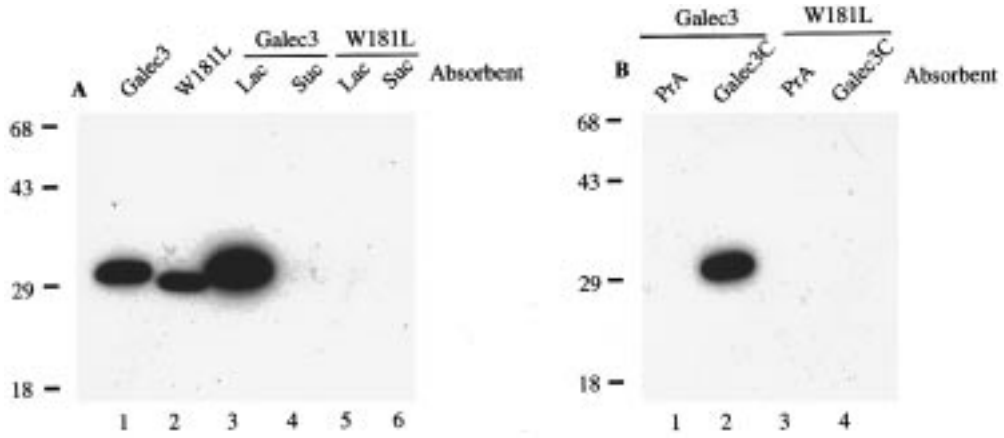

FIGURE 3: Evaluation of lactose-binding and self-association activities of galectin-3 mutant with tryptophan[181] replaced with leucine. (A) Lysates from Jurkat cell transfectants expressing galectin-3 or galectin-3(W181L) were mixed with lactosyl-Sepharose 4B (lanes 3 and 5) or sucrosyl-Sepharose 4B (lanes 4 and 6). The bound proteins were eluted and analyzed by immunoblotting. Lanes 1 and 2 contained the unabsorbed lysates from transfectants expressing galectin-3 and galectin-3(W181L), respectively. (B) Lysates from Jurkat transfectants expressing galectin-3 and galectin-3(W181L) were mixed with protein A−Sepharose 4B (lanes 1 and 3) or galectin-3C−Sepharose 4B (lanes 2 and 4). The bound proteins were eluted and analyzed by immunoblotting. The numbers on the left margin denote molecular weight ($\times 10^{-3}$) markers. PrA = protein A; galec3C = galectin-3C; W181L = galectin-3W(181L); Lac = lactosyl-Sepharose; Suc = sucrosyl-Sepharose.

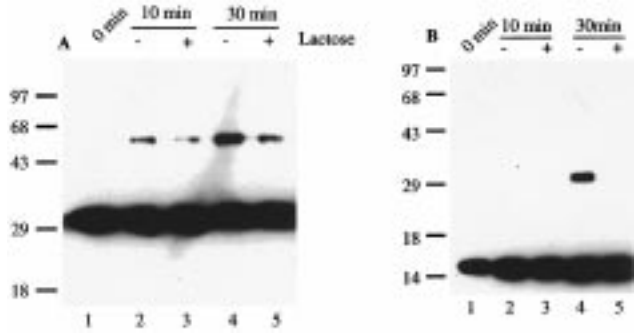

FIGURE 4: Demonstration of formation of stable dimers of galectin-3 and galectin-3C by chemical cross-linker. Galectin-3 (panel A) and galectin-3C (panel B) were treated with DMP for 10 (lanes 2 and 3) or 30 min (lanes 4 and 5) in the absence (lanes 2 and 4) or presence (lanes 3 and 5) of lactose. The reaction mixtures were analyzed by immunoblotting using anti-galectin-3 antibodies. Numbers on the left margin denote molecular weight ($\times 10^{-3}$) markers. Similar results were obtained from two separate experiments.

Galectin-3 is not a glycoprotein and thus the lactose-inhibitable self-association cannot be explained by the binding of the protein's lectin domain to saccharides on the same protein. Rather, the results suggest a peptide−peptide interaction that can take place only when the lectin's carbohydrate-binding site is not occupied. One intriguing possibility is that the carbohydrate-binding site is directly involved in the intermolecular interaction. However, we cannot exclude the possibility that another site is involved in the galectin-3 self-association and the occupancy of the carbohydrate-binding site causes a conformational change at this site, thus disallowing the association. Indeed, Agrwal et al. (1993) have provided evidence that lactose binding to galectin-3 induces a conformational change in the carboxyl-terminal domain of the lectin. The finding that the W181L mutant has lost both the lactose-binding activity and self-association property is in agreement with the involvement of the carbohydrate-binding site in self-association. However, we cannot exclude the possibility that the mutation of the tryptophan residue results in a global conformational

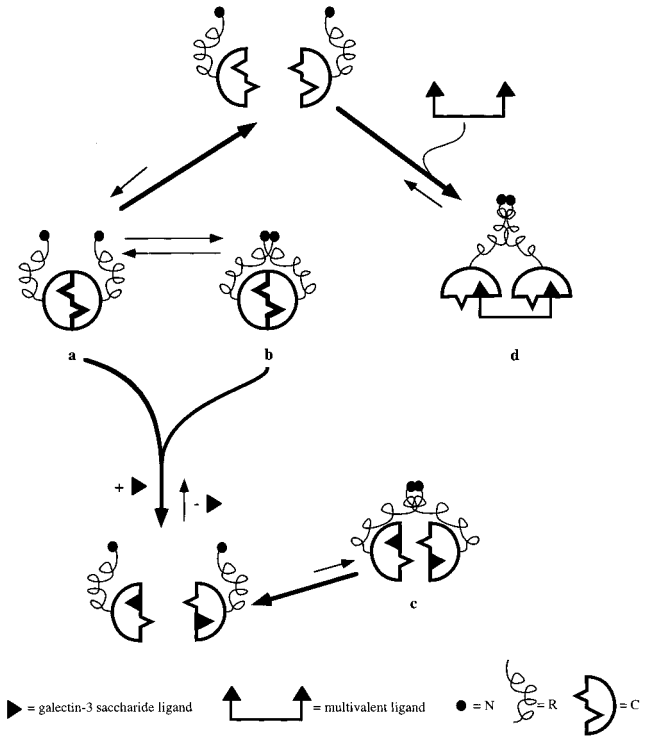

FIGURE 5: Schematic models for galectin-3 self-association. Galectin-3 undergoes self-association in the absence of saccharide ligands primarily mediated by the carboxyl-terminal domain (model a). The amino-terminal region interaction may also be involved (model b). In the presence of saccharide ligands, the carboxyl-terminal domain interaction is interrupted, but the protein can still self-associate by interaction between the amino-terminal regions (model c). The amino-terminal region is critical for self-association of galectin-3 that accompanies its binding to multivalent saccharide ligands (model d). In models b, c, and d, existing information does not allow specification of whether it is the amino-terminal domain, the tandem repeats, or both that are involved in self-association. Whether the carbohydrate-binding site is directly involved in the protein self-association, as depicted in models a and b, also requires clarification (see Discussion). While only homodimers are shown in the models, the existence of oligomeric interactions is not excluded. N = amino-terminal domain; R = tandem repeats; C = carboxyl-terminal domain.

change in galectin-3, thus affecting protein homodimerization, which may or may not involve this amino acid residue. We have attempted to address the question of whether there is a global conformational change in the W181L mutant. We have tried to purify sufficient amounts of GST fusion proteins of galectin-3C and galectin-3C(W181L), respectively, for comparison of their physicochemical properties. However, in significant contrast to the wild-type fusion protein, the mutant fusion protein could be obtained only in very low yields from the transformed *E. coli*, making it difficult to perform the studies. This finding alone might suggest that there is significant alteration in the protein as a result of a single amino acid mutation. On the other hand, it is to be noted that the levels of the two proteins are comparable in the stable transfectants of a mammalian cell line individually expressing these proteins. Additional studies are clearly needed to determine whether the carbohydrate-binding site is directly involved in galectin-3 self-association. Nevertheless, the results with the W181L mutant at least support the role of the carboxyl-terminal domain in galectin-3 self-association.

Our previous model is that, when bound to multivalent glycoconjugates, galectin-3 self-associates through intermolecular interactions involving the amino-terminal domain (Hsu et al. 1992; Liu, 1993). The present study revealed that, in the absence of saccharide ligands, the carboxyl-terminal domain is the major contributor to the galectin-3 self-association. First, the isolated carboxyl-terminal domain fragment can self-associate, as demonstrated by chemical cross-linking. Second, mutation in the carboxyl-terminal domain results in diminution of self-association. Third, inhibition of carboxyl-terminal domain interactions by lactose causes significant reduction in galectin-3 self-association. However, the present study also supports the contribution of the amino-terminal region. In particular, stable homodimer was still formed when the full-length galectin-3 was treated with chemical cross-linker in the presence of lactose, whereas homodimer formation of the carboxyl-terminal domain fragment was completely inhibited by lactose. These results are most consistent with a model in which both the amino-terminal region and carboxyl-terminal domain contribute to self-association and intermolecular interactions involving the latter but not the former are inhibitable by lactose. On one other hand, the binding of biotinylated galectin-3 to immobilized galectin-3 was nearly quantitatively inhibited by lactose, suggesting that the amino-terminal region alone is not sufficient to maintain protein self-association under this experimental condition. It is possible that when galectin-3 is adsorbed onto the plastic plates, the putative determinant(s) involved in the amino-terminal region interactions is(are) altered, and thus the carboxyl-terminal domain interaction then appears to be solely contributing to the binding (i.e., biotinylated galectin-3 to immobilized galectin-3).

Taking into consideration all the information generated in this and previous studies, we propose a revised model for galectin-3 self-association, which explains the multivalent behavior of galectin-3 and serves as an example that self-association of a protein can be modulated by the protein's binding to its ligand (Figure 5). In the absence of saccharide ligands, galectin-3 self-associates mainly through the carboxyl-terminal lectin domain (model a). The amino-terminal region may also contribute, possibly through intermolecular interactions between this region (model b). We envision that this carboxyl-terminal domain-mediated self-association may take place intracellularly, in which galectin-3 is not bound to glycoconjugates, and may be related to the lectin's intracellular functions, e.g., regulation of cell growth and apoptosis (Yang et al. 1996). It is important to point out that galectin-3 exists primarily as monomers in solution, suggesting that the intermolecular interactions involved in the self-association proposed in our model are rather weak interactions. In the presence of saccharide ligands, the carboxyl-terminal domain interaction is interrupted, but the protein can still self-associate through amino-terminal region interactions (model c). Model d represents the situation in which galectin-3 encounters multivalent ligands and the protein forms dimers or oligomers through amino-terminal region interactions, as proposed earlier (Hsu et al. 1992). It is possible that when the lectin interacts with its saccharide ligands, the amino-terminal region undergoes a conformational change, thus becoming more favorably predisposed to self-association. Such a putative conformational change may result in a configuration that resembles the isolated amino-terminal fragment, which has been shown to have a higher tendency to self-associate than the intact lectin (Mehul et al. 1994).

ACKNOWLEDGMENT

We thank Dr. Shigekazu Nagata at Osaka Bioscience Institute, Japan and Dr. Yun-Cai Liu at La Jolla Institute for Allergy and Immunology for providing plasmids pEF-BOS and pEF-Neo, respectively.

REFERENCES

Agrwal, N., Sun, Q., Wang, S.-Y., & Wang, J. L. (1993) *J. Biol. Chem. 268*, 14932.

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

Baenziger, J., Kornfeld, S., & Kochwa, S. J. (1974) *J. Biol. Chem. 249*, 1897.

Bao, Q., & Hughes, R. C. (1995) *J. Cell Sci. 108*, 2791.

Barondes, S. H., Cooper, D. N. W., Gitt, M. A., & Leffler, H. (1994) *J. Biol. Chem. 269*, 20807.

Cherayil, B. J., Weiner, S. J., & Pillai, S. (1989) *J. Exp. Med. 170*, 1959.

Cherayil, B. J, Chaitovitz, S., Wong, C., & Pillai, S. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 7324.

Dagher, S. F., Wang, J. L., & Patterson, R. J. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 1213.

Dyer, K. D., Handen, J. S., & Rosenberg, H. F. (1997) *Genomics 40*, 217.

Frigeri, L. G., Robertson, M. W., & Liu, F.-T. (1990) *J. Biol. Chem. 265*, 20763.

Frigeri, L. G., Zuberi, R. I., & Liu, F.-T. (1993) *Biochemistry 32*, 7644.

Gitt, M. A., Wiser, M. F., Leffler, H., Herrmann, J., Xia, Y.-R., Massa, S. M., Cooper, D. N. W., Lusis, A. J., & Barondes, S. H. (1995) *J. Biol. Chem. 270*, 5032.

Hadari, Y. R., Paz, K., Dekel, R., Mestrovic, T., Accili, D., & Zick, Y. (1995) *J. Biol. Chem. 270*, 3447.

Hamann, K. K., Cowles, E. A., Wang, J. L., & Anderson, R. L. (1991) *Exp. Cell Res. 196*, 82.

Hanada, M., Aimé-Sempé, C., Sato, T., & Reed, J. C. (1995) *J. Biol. Chem. 270*, 11962.

Herrmann, J., Turck, C. W., Atchinson, R. E., Huflejt, M. E., Poulter, L., Gitt, M. A., Burlingame, A. L., Barondes, S. H., & Leffler, H. (1993) *J. Biol. Chem. 268*, 26704.

Hsu, D. K., Zuberi, R., & Liu, F.-T. (1992) *J. Biol. Chem. 267*, 14167.
Jia, S., & Wang, J. L. (1988) *J. Biol. Chem. 263*, 6009.
Kasai, K., & Hirabayashi, J. (1996) *J. Biochem. (Tokyo) 119*, 1.
Kuwabara, I., & Liu, F.-T. (1996) *J. Immunol. 156*, 3939.
Levi, G., & Teichberg, V. I. (1981) *J. Biol. Chem. 256*, 5735.
Liao, D.-I., Kapadia, G., Ahmed, H., Vasta, G. R., & Herzberg, O. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 1428.
Liu, F.-T., Bohn, J. W., Ferry, E. L., Yamamoto, H., Molinaro, C. A., Sherman, L. A., Klinman, N. R., & Katz, D. H. (1980) *J. Immunol. 124*, 2728.
Liu, F.-T. (1993) *Immunol. Today 14*, 486.
Liu, F.-T., & Orida, N. (1984) *J. Biol. Chem. 259*, 10649.
Liu, F.-T., Hsu, D. K., Zuberi, R. I., Kuwabara, I., Chi, E. Y., & Henderson, W. R., Jr. (1995) *Am. J. Pathol. 147*, 1016.
Liu, F.-T., Hsu, D. K., Zuberi, R. I., Shenhav, A., Hill, P. N., Kuwabara, I., & Chen, S.-S. (1996) *Biochemistry 35*, 6073.
Lobsanov, Y. D., Gitt, M. A., Leffler, H., Barondes, S. H., & Rini, J. M. (1993) *J. Biol. Chem. 268*, 27034.
Madsen, P., Rasmussen, H. H., Flint, T., Gromov, P., Kruse, T. A., Honoré, B., Vorum, H., & Celis, J. E. (1995) *J. Biol. Chem. 270*, 5823.
Massa, S. M., Cooper, D. N. W., Leffler, H., & Barondes, S. H. (1993) *Biochemistry 32*, 260.
Mehul, B., Bawumia, S., Martin, S. R., & Hughes, R. C. (1994) *J. Biol. Chem. 269*, 18250.
Mizushima, S., & Nagata, S. (1990) *Nucleic Acids Res. 18*, 5322.
Ochieng, J., Platt, D., Tait, L., Hogan, V., Raz, T., Carmi, P., & Raz, A. (1993) *Biochemistry 32*, 4455.
Patel, R. C., Stanton, P., McMillan, N. M., Williams, B. R., & Sen, G. C. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 8283.
Raz, A., Pazerini, G., & Carmi, P. (1989) *Cancer Res. 49*, 3489.
Raz, A., Zhu, D., Hogan, V., Shah, N., Raz, T., Karkash, R., Pazerini, G., & Carmi, P. (1990) *Int. J. Cancer 46*, 871.
Raz, A., Carmi, P., Raz, T., Hogan, V., Mohamed, A., & Wolman, S. R. (1991) *Cancer Res. 51*, 2173.
Robertson, M. W., Albrandt, K., Keller, D., & Liu, F.-T. (1990) *Biochemistry 29*, 8093.
Truong, M.-J., Gruart, V., Kusnierz, J.-P., Papin, J.-P., Loiseau, S., Capron, A., & Capron, M. (1993) *J. Exp. Med. 177*, 243.
Tsujimura, T., Koshimizu, U., Katoh, H., Isozaki, K., Kanakura, Y., Tono, T., Adachi, S., Kasugai, T., Tei, H., Nishimune, Y., Nomura, S., & Kitamura, Y. (1993) *Blood 81*, 2530.
Uetsuki, T., Naito, A., Nagata, S., & Kaziro, Y. (1989) *J. Biol. Chem. 264*, 5791.
Voss, P. G., Tsay, Y.-G., & Wang, J. L. (1994) *Glycoconjugate J. 11*, 353.
Wada, J., & Kanwar, Y. S. (1997) *J. Biol. Chem. 272*, 6078.
Yamaoka, A., Kuwabara, I., Frigeri, L. G., & Liu, F.-T. (1995) *J. Immunol. 154*, 3479.
Yang, R.-Y., Hsu, D. K., & Liu, F.-T. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 6737.
Zuberi, R. I., Frigeri, L. G., & Liu, F.-T. (1994) *Cell Immunol. 156*, 1.

BI971409C