Quaternary solution structures of galectins-1, -3, and -7

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Galectins are a growing family of animal lectins with functions in growth regulation and cell adhesion that bind β-Gal residues in oligosaccharides. Evidence indicates that some of the biological properties of galectins are due to their cross-linking activities with multivalent glycoconjugate receptors. Therefore determination of the quaternary solution structures of these proteins is important in understanding their structure-function properties. The present study reports analytical sedimentation velocity and equilibrium data for galectins-1, -3, and -7 in the absence and presence of bound LacNAc, the natural ligand epitope. Galectin-1 from bovine heart and recombinant human galectin-7 were found to be stable dimers by both methods. In contrast, recombinant murine galectin-3, as well as its proteolytical derived C-terminal domain, are predominantly monomeric. The presence of LacNAc at concentrations sufficient to fully saturate the proteins had no significant effect on either the weight average molecular weight determined by sedimentation equilibrium or the hydrodynamic properties determined from sedimentation velocity experiments. These results show that binding of a multivalent ligand does not affect oligomerization of these galectins.

Key words: galectin-1/galectin-3/galectin-7/quaternary structures

Introduction

Galectins are a family of animal lectins with common consensus sequences and structures that specifically bind β-Gal and LacNAc residues in oligosaccharides (Kasai and Hirabayashi, 1996; Liu, 2000; Lobsanov and Rini, 1997). Fourteen members of the family have been identified in family members are likely to be discovered because homodimers consisting of one carbohydrate-recognition domain (CRD) per subunit; the chimera type (galectin-3), which contains a nonlent C-terminal short sequence segment followed by 8–12 collagen-like repeats of 9 amino acids connected to the C-terminal CRD domain; and tandem-repeat type (galectins-4, -6, -8, -9, and -12) composed of two CRD domains in a single polypeptide chain connected by a linker peptide (Leffler, 1997; Yang et al., 2001).

Insight into the biological activities of specific galectins has grown in recent years. Galectin-1 induces apoptosis of human thymocytes, activated T cells, and T lymphoblastoid cell lines (e.g., Perillo et al., 1998). In the thymus, thymic epithelial cells produce galectin-1. Evidence suggests that galectin-1 eliminates antigen-specific T cells, implying a role in terminating an immune response in the periphery (Perillo et al., 1995). Galectin-1 has also been implicated in inflammation, development, mRNA splicing, differentiation, cell adhesion, and metastasis (e.g., Liu, 2000). Galectin-3, unlike galectin-1, possesses antiapoptotic effects in a variety of cells (e.g., Rabinovich et al., 2002). Its expression has been shown to correlate with the metastatic potentials of certain cancers (e.g., Akahani et al., 1997). Galectin-3 also contains a functional BH1 (NWGR) region of the Bcl-2 family of proteins that is associated with their antiapoptotic activities (Akahani et al., 1997). The 28% identity and 48% similarity between the sequences of galectin-3 and Bcl-2 suggests the conservation of certain activities by the two proteins (Yang et al., 1996). Galectin-3 has also been shown to be involved in regulating inflammation, cell growth, and cell adhesion (Liu, 2000). In a neuroblastoma model, galectin-3 has been shown to block galectin-1-mediated growth inhibition on the level of ligand binding (Kopitz et al., 2001).

The galectin-7 gene is a transcriptional target of tumor suppressor protein p53 and may play a role in the proapoptotic function of p53 (Polyak et al., 1997). Galectin-7 also increases the susceptibility of keratinocytes to UVB-induced apoptosis (Bernerd et al., 1999). In addition, galectin-7 is a marker for stratified epithelia (Magnaldo et al., 1998) and is elevated in chemically induced rat mammary carcinomas (Lu et al., 1997). Its frequent gene expression in human breast and colon cancer cell lines suggests a possible role in malignancy (Lu et al., 1997). Thus galectins-1, -3, and -7 are implicated in regulating cell survival activities. Evidence exist that other members of the galectin family have similar roles (Liu, 2000).

To understand the molecular basis of the differences in the biological activities of galectins-1, -3, and -7, the binding
specificities of the three lectins need to be well understood. Indeed, a comparative study of the carbohydrate binding specificities of galectins-1, -3, and -7 using isothermal titration microcalorimetry (Ahmad et al., 2002) has recently shown important differences in their carbohydrate binding specificities. In particular, galectins-3 and -7 were demonstrated to bind to nonreducing terminal and internal LacNAc residues of the poly-N-acetyllactosamine chains that are often found on glycoprotein receptors (e.g., Pace et al., 1999). In contrast, galectin-1 was shown to bind primarily to terminal LacNAc residues on such chains.

The relationship between the quaternary structures of galectins-1, -3, and -7 in solution and their biological activities is also important because of their multivalent carbohydrate binding and cross-linking properties (e.g., Lobanov and Rini, 1997). The present study reports sedimentation velocity and equilibrium analytical ultracentrifugation studies of bovine heart galectin-1, recombinant murine galectin-3 and its isolated CRD domain, and recombinant human galectin-7.

The results provide direct determinations of the quaternary structures in solution of these galectins in the absence and presence of bound monovalent carbohydrate. These findings are important for understanding the relationship between the quaternary structures of these galectins and their biological activities.

Results

Sedimentation velocity

Representative boundary scans of galectins-1, -3, and -7 as the protein solution sediments to the bottom of the cells are shown in Figure 1. Each centrifuge cell consists of two sectors, one containing solvent and the other containing the solution allowing the sedimentation of the protein to be followed by absorption of 280 nm light. The solution is run at high angular velocities such that it sediments fairly quickly to the cell bottom. This sedimentation produces a sharp boundary between the region of depleted solute and that of the concentration of sedimenting solute. From these boundaries the rates that the proteins sediment and diffuse are determined.

For each galectin, a single symmetric boundary was detected indicating that the solutions are homogeneous. Comparable results were observed for the galectins to which an excess of LacNAc was added. This behavior of the boundaries is summarized in the plots of $dC/dt$ versus $S_{20,w}$ for the liganded and unliganded proteins shown in Figure 2. The apparent molecular weight of galectin-3 calculated from the ratio $S_{20,w}/D_{20,w}$ is that of the protein monomer within experimental error (Table I). In contrast, both galectin-1 and galectin-7 appear to be dimeric by the same criteria (Table I). The sedimentation behavior of the proteins does not significantly change in the presence of saturating concentrations of LacNAc. The sedimentation velocity data acquired for the three galectins are well described by the single component model in both the presence and absence of LacNAc (Figure 2, Table I).

The values of $S_{20,w}$ and $D_{20,w}$ determined as a function of galectin concentration are consistent with galectins-1 and -7 being stable dimers because these values do not appreciably increase (Figure 3, squares and diamonds). The apparent molecular weights calculated from the ratio $S_{20,w}/D_{20,w}$, obtained by extrapolating these data to infinite dilution, are within experimental error of the molecular weights determined from the protein’s sequence (Table I). Only for galectin-3 does $S_{20,w}$ increase with protein concentration, a behavior characteristic of self-association (Figure 3, circles).

To further probe possible self-association of galectin-3, the carboxyl region (galectin-3 CRD) of this protein was similarly examined. $S_{20,w}$ did not increase with increasing concentration of the truncated protein (data not shown). The values of $S_{20,w}$ of 1.76 $S$ and $D_{20,w}$ of 10.4 $f$ determined for the truncated protein yield an apparent molecular weight (from $S_{20,w}/D_{20,w}$) of 15.32 ± 0.27 kDa, which is within the experimental error of that determined from the protein sequence.

Sedimentation equilibrium

Sedimentation equilibrium experiments were conducted with galectins-1, -3, and -7 in the absence and presence of LacNAc to determine the weight average molecular
weight ($M_w$) of the proteins in a thermodynamically rigorous manner (Figure 4; Table I). Galectin-3 is satisfactorily fit as a single component yielding a value of $M_w$ that is within error of the molecular weight calculated from sequence for a protein monomer. In contrast, both galectin-1 and -7 yield values of $M_w$ consistent with protein dimers. The fits of these data to the single component model are characterized by small, uniformly distributed residuals. In contrast, fits of the data to self-association models (i.e., monomer–dimer) either failed to converge or yielded nonrandom residual distributions and elevated square roots of the variance (results not shown).

The good fits of the single species model to the galectin-1 data (Figure 4) and the slight elevation of the value of $M_w$ determined for this protein compared to that calculated from sequence (Table I) suggests that galectin-1 undergoes self-association at protein concentrations higher than those studied herein. Our inability to characterize this postulated assembly reaction is due to the minimal concentrations of oligomers present at the highest concentrations of protein studied.

The conclusions from these data are that galectin-3 is monomeric and that galectins-1 and -7 are dimeric under the conditions of these experiments. The quaternary structures of these proteins are not affected by binding LacNAc (Figure 4; Table I). In addition, examination of the values of $S_{20,w}$ and $D_{20,w}$ in the context of the independently determined molecular weights suggests that the global conformation of these proteins are well described by uniform sphere, and thus not appreciably asymmetric, and are unaffected by the binding of LacNAc (Figure 2; Table I).

Discussion

Evidence indicates that the multivalent binding properties of galectins play central roles in their interactions with multivalent glycoconjugate receptors (e.g., Demetriou et al., 2001; Dennis et al., 2001; Pace et al., 1999; Sacchettini et al., 2001). Determination of the quaternary structures of this family of protein therefore is important in understanding their structure–function properties. However, to our knowledge there have been no detailed reports of the hydrodynamic properties of galectins and their solution quaternary structures using sedimentation velocity and equilibrium analytical ultracentrifugation measurements.

Binding studies, including hemagglutination measurements of galectin-1, suggest that it is an oligomeric protein (Barondes et al., 1994). Subsequent X-ray diffraction studies of bovine galectin-1 in the presence of LacNAc (Liao et al., 1994) and in several different cross-linked complexes with a biantennary N-glycan (Bourne et al., 1994) revealed the crystallized protein to be a symmetrical dimer. Mutagenesis studies of galectin-1 from Chinese hamster ovarian cells yielded monomeric protein variants with reduced affinity toward polyvalent carbohydrates (e.g., Cho and
Galectin-1 (\(M_w\) of dimer from sequence) is dimeric over the concentration range tested. It is important to emphasize that sedimentation equilibrium is a true thermodynamic method that is insensitive to the shape of macromolecules. The addition of LacNAc, a monovalent carbohydrate, does not change the quaternary structure of the protein as evidenced by the consistency of \(S_{20,w}\) and \(D_{20,w}\) in sedimentation velocity experiments conducted in the presence and absence of bound ligand. The absence of appreciable increases in \(S_{20,w}\) and decreases in \(D_{20,w}\) with galectin-1 concentration is additional evidence for the stability of the dimer over this concentration range of protein concentrations. These results further suggest that the dimeric structure of galectin-1 observed in the protein's crystal structure is an appropriate model for the protein's structure in solution.

Galectin-7 has also been reported to be a dimer in the crystalline state (Leonidas et al., 1998). However, other reports indicate that galectin-7 is monomeric in solution (Cooper and Barondes, 1999; Leffler, 1997; Leonidas et al., 1998). The molecular weight of galectin-7 as determined by these ultracentrifugation and sedimentation experiments (Table I) shows that it is also a dimer in solution, consistent with the dimer present in the crystal structure of the protein (Leonidas et al., 1998), and the hemagglutination activity of the lectin (Ahmad et al., 2002). Neither the sedimentation velocity or equilibrium data provide evidence for further self-association of galectin-7 over the range of protein concentrations examined.

Unlike other galectins including galectins-1 and -7, galectin-3 is comprised of two different domains: a C-terminal CRD domain and an N-terminal domain consisting of tandem repeats of proline- and glycine-rich motifs. The number of tandem repeats is species-dependent. The isolated N-terminal domain has been shown to self-associate (Mehul et al., 1994). The X-ray crystal structure of the C-terminal CRD domain of galectin-3 (Seetharaman et al., 1998) is not a dimer as observed for galectins-1 and -2 (Lobsanov and Cummings, 1995, 1997) and reduced biological activity (Pace et al., 1999). Although size exclusion chromatography suggests that the galectin-1 dimer from Chinese hamster ovary cells is in dynamic equilibrium with its monomer at micromolar concentrations (Cho and Cummings, 1995), such studies do not provide accurate molecular mass determinations (see Figure 5 of Cho and Cummings, 1995) because of the tendency of lectins to bind to the matrices of such columns (Brewer, unpublished data). Hence, direct determination of the quaternary structure of mammalian galectin-1 \textit{in solution} has not been reported.

The sedimentation equilibrium and velocity results summarized in Table I demonstrate that bovine galectin-1 is dimeric over the concentration range tested. It is important to emphasize that sedimentation equilibrium is a true thermodynamic method that is insensitive to the shape of macromolecules. The addition of LacNAc, a monovalent carbohydrate, does not change the quaternary structure of the protein as evidenced by the consistency of \(S_{20,w}\) and \(D_{20,w}\) in sedimentation velocity experiments conducted in the presence and absence of bound ligand. The absence of appreciable increases in \(S_{20,w}\) and decreases in \(D_{20,w}\) with galectin-1 concentration is additional evidence for the stability of the dimer over this concentration range of protein concentrations. These results further suggest that the
dimeric structure of galectin-1 observed in the protein’s crystal structure is an appropriate model for the protein’s structure in solution.

### Table I. Sedimentation velocity and equilibrium data and analysis for galectins-1, -3, and -7 in the absence and presence of bound saccharide

<table>
<thead>
<tr>
<th>LacNAc (mM)</th>
<th>Galectin (µM)</th>
<th>(S_{20,w}) (S)</th>
<th>(D_{20,w}) (f)</th>
<th>(S_{20,w}D_{20,w}) (kDa)</th>
<th>(M_w) (KDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Galectin-1 ((M_w) of dimer from sequence)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>32–95</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>29.2</td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>2.55 ± 0.01</td>
<td>8.86 ± 0.90</td>
<td>27.07 ± 2.66</td>
<td>33.49 (30.80, 36.14)</td>
</tr>
<tr>
<td>0.0</td>
<td>69</td>
<td>2.56 (2.53, 2.58)</td>
<td>7.62 (6.58, 8.36)</td>
<td>30.13 (26.68, 34.13)</td>
<td>—</td>
</tr>
<tr>
<td>6.0</td>
<td>32–95</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>31.24 (28.16, 34.30)</td>
</tr>
<tr>
<td>21.0</td>
<td>69</td>
<td>2.59 (2.57, 2.61)</td>
<td>9.73 (8.68, 10.40)</td>
<td>23.92 (21.93, 26.33)</td>
<td>—</td>
</tr>
<tr>
<td><strong>Galectin-3 ((M_w) of monomer from sequence)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>15–45</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>27.4</td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>2.10 ± 0.03</td>
<td>7.00 ± 0.26</td>
<td>27.09 ± 1.27</td>
<td>—</td>
</tr>
<tr>
<td>0.0</td>
<td>31</td>
<td>2.209 (2.19, 2.23)</td>
<td>6.90 (5.97, 7.52)</td>
<td>28.77 (25.78, 32.57)</td>
<td>—</td>
</tr>
<tr>
<td>6.0</td>
<td>15–45</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>26.66 (25.19, 28.09)</td>
</tr>
<tr>
<td>14.0</td>
<td>31</td>
<td>2.258 (2.24, 2.28)</td>
<td>6.55 (5.89, 7.19)</td>
<td>30.96 (27.47, 34.28)</td>
<td>—</td>
</tr>
<tr>
<td><strong>Galectin-7 ((M_w) of dimer from sequence)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>31–93</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>29.8</td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>2.47 ± 0.01</td>
<td>8.09 ± 0.90</td>
<td>28.47 ± 1.08</td>
<td>—</td>
</tr>
<tr>
<td>0.0</td>
<td>56</td>
<td>2.465 (2.45, 2.48)</td>
<td>7.60 (6.61, 8.30)</td>
<td>29.57 (27.23, 32.12)</td>
<td>—</td>
</tr>
<tr>
<td>16</td>
<td>31–93</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>29.36 (27.12, 31.59)</td>
</tr>
<tr>
<td>16</td>
<td>54</td>
<td>2.516 (2.50, 2.54)</td>
<td>7.79 (6.36, 8.48)</td>
<td>29.45 (27.10, 32.96)</td>
<td>—</td>
</tr>
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</table>

*The molecular weight (\(M_w\)) tabulated for sedimentation velocity experiments is calculated from the ratio of the sedimentation and diffusion constants (\(S_{20,w}/D_{20,w}\)). The weight average molecular weights determined from sedimentation equilibrium experiments conducted as described in \textit{Materials and methods} determined from the data shown in Figure 4. The concentration range indicates the initial maximum and minimum galectin concentrations.

The molecular weight values shown were determined from models assuming a stable monomer (galectin-3) or stable dimer (galectins-1 and -7) as described in the text. The values in brackets indicate the 95% confidence intervals determined from the nonlinear least-squares fits of the data.

The values of \(S_{20,w}\) and \(D_{20,w}\) were determined by extrapolation of the data shown in Figure 3 to infinite dilution.

*These sedimentation velocity experiments were conducted at 35,000 rpm and correspond to the plots shown in Figure 2. The values in brackets indicate the 95% confidence intervals determined from the nonlinear least-squares fits of the data to a single component model.
and Rini, 1997). Inspection of the crystal structure of the galectin-3 CRD domain reveals an apolar patch in the face of the 5-stranded β-sheet that is suggested to provide a putative monomer–monomer interface (Seetharaman et al., 1998).

There is considerable ambiguity in the literature regarding the quaternary structure of galectin-3. There are reports using size-exclusion chromatography that galectin-3 is either monomeric (Massa et al., 1993) or dimeric (Ochieng et al., 1993). Galectin-3 is also reported to be dimeric under nonreducing conditions using sodium dodecyl sulfate (SDS) gel chromatography (Woo et al., 1991). Galectin-3 is reported to be chemically cross-linked at relatively high monomer concentrations to form dimers and higher molecular weight aggregates (Hsu et al., 1992). Transglutaminase is reported to cross-link galectin-3 into dimers (Mehul et al., 1995), and solid-phase binding data is said to be consistent with galectin-3 being a dimer (Kuklinski and Probstmeier, 1998). Thus using a variety of indirect methods the quaternary structure of galectin-3 has been reported to be a monomer, dimer, or higher oligomer.

There is also uncertainty in the relationship between the quaternary structure of galectin-3 and its carbohydrate binding properties. A previous study reported that galectin-3 exhibits positive cooperativity on binding to immobilized laminin and concluded that binding to a substrate surface induced aggregation of the lectin (Massa et al., 1993). Other reports indicate that galectin-3 aggregates on binding multivalent glycoconjugates and that the N-terminal domain of galectin-3 as well as the C-terminal domain are involved in the aggregation and positive binding cooperativity of the lectin (e.g., Yang et al., 1998).

To directly determine the quaternary structure of galectin-3, sedimentation velocity and equilibrium studies were performed with the protein in the absence and presence of LacNAC, a monovalent sugar. The molecular mass of galectin-3 determined by sedimentation velocity and equilibrium experiments (Table I) indicates that the protein is predominantly monomeric in solution at the concentrations analyzed. However, the increase in $S_{20,w}$ coupled with the decrease in $D_{20,w}$ observed in sedimentation velocity experiments as a function of galectin-3 concentration suggests that this protein does oligomerize to a small extent with increasing concentrations.

There are several reports proposing that the N-terminal domain is responsible for galectin-3 self-association (e.g., Hsu et al., 1992; Mehul et al., 1994). That $S_{20,w}$ and $D_{20,w}$ are not appreciably dependent on the concentration of the C-terminal CRD domain of galectin-3 is consistent with the hypothesis that the N-terminal domain is responsible for the association of galectin-3 in solution. This result is consistent with the correlate that the C-terminal CRD domain does not possess intrinsic self-association activity, as previously suggested (e.g., Kuklinski and Probstmeier, 1998).

The presence of bound LacNAC did not change either the molecular weight or the hydrodynamic parameters of intact galectin-3 or its CRD domain (Table I). These results indicate that galectin-3 neither oligomerizes nor grossly changes conformation on binding a monovalent sugar. These findings are important in terms of understanding the mechanism of cross-linking and precipitation of certain synthetic multivalent carbohydrates by galectin-3 (Brewer et al., unpublished data).

The results of the present study may also be important in terms of understanding the biological activities of galectin-1, -3, and -7. Galectins-1 and -7 are reported to possess apoptotic activities in certain cells, whereas galectin-3 has been shown to possess antiapoptotic activities in certain cells (Rabinovich et al., 2002). Moreover, the competitive inhibition of the galectin-1 effect on neuroblastoma cells by galectin-3 gives reason to link galectin mediated cross-linking to cell signal transduction mechanisms (Kopitz et al., 2001). The fact that galectins-1 and -7 are dimeric proteins in solution and galectin-3 is predominantly a monomer indicates that the quaternary structures of these proteins may relate to their different biological properties.

**Materials and methods**

**Materials**

LacNAc type II was obtained from Sigma (St. Louis, MO). All other reagents were of analytical grade. Galectin-1 from bovine heart was purified using the procedure for the
isolation of calf spleen galectin-1 (Gupta and Brewer, 1994). SDS–polyacrylamide gel electrophoresis (PAGE) of the affinity-purified lectin was found to contain one more additional band. Affinity-purified lectin was fractionated into two clear peaks, I and II, by gel chromatography using Sephadex G-50 column in the presence of 0.05 mM lactose. The second peak corresponded to galectin-1 and was found to be pure by SDS–PAGE. Fractions containing the second peak were pooled and dialyzed extensively against phosphate buffered saline containing 10 mM β-mercaptoethanol and stored at 4°C.

The plasmid prCBP35s with cDNA for murine galectin-3 was a gift from John L. Wang (East Lansing), and the protein was expressed as described in the literature with proteolytic degradation by a collagenase, yielding truncation of the sensitive N-terminal region (Agrwal et al., 1993). Recombinant human galectin-7 was expressed using the plasmid pQE-60/hGal-7 kindly provided by F.-T. Liu (Davis) as described (André et al., 2001; Kuwabara et al., 2002). All of the proteins showed single bands by SDS–PAGE.

Monomeric lectin concentrations were determined spectrophotometrically at 280 nm using the specific extinction coefficient of each protein (E1%1cm). A value of 5.4 (E1%1cm) was used for galectin-1, 6.1 (E1%1cm) for galectin-3, and 5.5 (E1%1cm) for galectin-7 as determined from ITC experiments. Molecular masses of the galectins were determined by matrix-assisted laser desorption/ionization and electrospray ionization mass spectrometry and were found to be 14,500 Da for galectin-1, 27,500 Da for galectin-3, and 14,600 Da for galectin-7.

**Analytical ultracentrifugation**

Sedimentation velocity experiments were performed using a Beckman Optima XL-I analytical ultracentrifuge, Al-Epon double-sector centerpieces, and the AN-60Ti rotor. Velocity experiments were performed at 35,000 rpm and 20°C with the sedimentation boundaries scanned at 280 nm using the absorption optics. Both the samples and rotor were temperature equilibrated prior to initiating each run. Monomeric galectin concentrations were determined spectrophotometrically at 280 nm using the above extinction coefficients of each protein. Galectins-1, -3, and -7 were each centrifuged in 20 mM sodium phosphate, 0.15 M NaCl, and 10 mM β-mercaptoethanol at pH 7.2 in the presence and absence of ligand (LacNAc). The sedimentation boundaries were directly fit as the derivative dC/dt using Svedberg v6.39 (Philo, 1994) to determine the sedimentation and diffusion coefficients, s and D, respectively, or s and the apparent molecular weight, Mw,app. Generally 20–30 absorbance scans were globally analyzed for each experiment. The observed values were normalized to standard conditions of 20°C and water (s20,w and D20,w) by correcting for buffer density and viscosity.

Sedimentation equilibrium experiments were performed at 20°C in the buffer described using either the AN-50Ti or
AN-60Ti rotors. Galectins-1 and 3 were analyzed in the presence and absence of 6.24 mM of its ligand, LacNAC. Galectin-7 was analyzed in the presence and absence of 16 mM LacNAC. For each condition, data were collected at three concentrations ($A_{280} = 0.3, 0.5,$ and 0.9) and two rotor speeds (15,000 rpm and 20,000 rpm). Monomeric concentrations were determined by using the extinction coefficients of each galectin as discussed. Absorbance scans at 280 nm were taken after 22 and 24 h at speed; equilibrium was assumed to have been reached if the scans were unchanged. Typically two scans were taken at 24 h at each rotor speed. Data analysis was performed using the Beckman XL-A/ XL-I data analysis software package within Microcal, ORIGIN v4, using values of the buffer density and protein partial specific volume determined as will be described. Each analysis consists of the six absorbance scans taken of the three different nominal concentrations at each of the two rotor speeds.

Buffer densities were determined using a Mettler DE40 density meter operated at the experimental temperature as well as in the program Sednterp v1.03. Partial specific volume was determined from amino acid residue concentrations at each of the two rotor speeds. Data analysis was performed using the Beckman XL-A/ XL-I data analysis software package within Microcal, ORIGIN v4, using values of the buffer density and protein partial specific volume determined as will be described. Each analysis consists of the six absorbance scans taken of the three different nominal concentrations at each of the two rotor speeds.

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Abbreviations
CRD, carbohydrate-recognition domain; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

References


