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Kinetic measurements of binding of galectin 3 to a laminin substratum

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Galectin 3, a β-galactoside binding protein, contains a C-terminal carbohydrate recognition domain (CRD) and an N-terminal segment including multiple repeats of a proline/tyrosine/glycine-rich motif. Previous work has shown that galectin 3 but not the isolated CRD binds to laminin, a multivalent ligand, with positive cooperativety indicating the formation of multiple interactions although the lectin in solution is monomeric. Using surface plasmon resonance, we find that hamster galectin 3 at sub-μmolar concentrations or its isolated CRD at all concentrations binds to a laminin substratum with similar association (k_{ass}; 10 − 30 000 M^{-1} S^{-1}) and dissociation (k_{diss}; 0.2 − 0.3 S_1^{-1}) rates and weak affinity (Ka; 1 − 3 × 10^5 M^{-1}). At higher concentrations of galectin 3 the off rate decreases ten fold leading to increased affinity. Ligation of an N-terminal epitope of galectin 3 with a monoclonal Fab fragment increases association and dissociation rates ten fold. A recombinant protein obtained by deletion of the first 93 N-terminal residues binds to laminin with positive cooperativity and a slowly dissociating fraction (K_{diss}; 0.002 S^{-1}) accumulates on the substratum. The data suggest that homophilic interactions between CRD as well as N terminal domains are implicated in galectin 3 aggregation on the substratum leading to positive binding cooperativity.

Keywords: **galectin 3, laminin binding, kinetics, cooperativity**

Introduction

Galectin-3, a member of a family of mammalian β-galactoside binding proteins [1–4], is expressed on various inflammatory cells and on many epithelial cells. It promotes homotypic cell-cell adhesion as well as cell-substratum adhesion [3,4] and is an important modulator of cellular activity. It participates in the allergic response of basophilic cells by binding to IgE and Fc receptors to stimulate degranulation and serotonin release [5], induces an oxidative burst in human neutrophils or eosinophils [6] and potentiates interleukin-1 production and release by human monocytes [7]. All of these biological effects suggest that galectin-3 can bind specifically to biologically relevant receptors and mediate multivalent binding interactions. Such interactions are needed to link cell-adhesion molecules on the surface of one cell with those on another cell or within the extracellular matrix to enhance adhesion, and in other systems are required to induce cross-linking of cell surface receptors leading to a biological response.

Previous studies have shown that galectin-3 binds with positive cooperativity to substrate-immobilized ligands including laminin [8], IgE [9] and myelin-associated glycoprotein MAG [10]. Although this property implies multivalency in binding, the mechanism by which this is achieved is not clear at present. Galectin-3 contains a single carboxyl-terminal carbohydrate recognition domain (CRD), homologous in three-dimensional structure to the CRDs of other galectins [11,12], and a unique N-terminal domain including tandem repeats of proline-glycine-tyrosine-glutamine rich motifs (Fig. 1A). This domain constitutes a flexible moiety with little secondary structure as shown by biophysical measurements and by extreme susceptibility to collagenases [9,13-16]. Under normal conditions in solution galectin-3 appears to be monomeric as assessed by gel-permeation chromatography [8,9,17]. However, small amounts of non-covalently bonded dimeric and higher species have been detected at relatively high concentrations [16,17] that could be captured by chemical or enzymic cross-linking [9,14,18,19]. These findings suggest that at least some transient association of lectin sub-units can occur in solution providing one possible explanation for cooperative binding. However, alternative mechanisms

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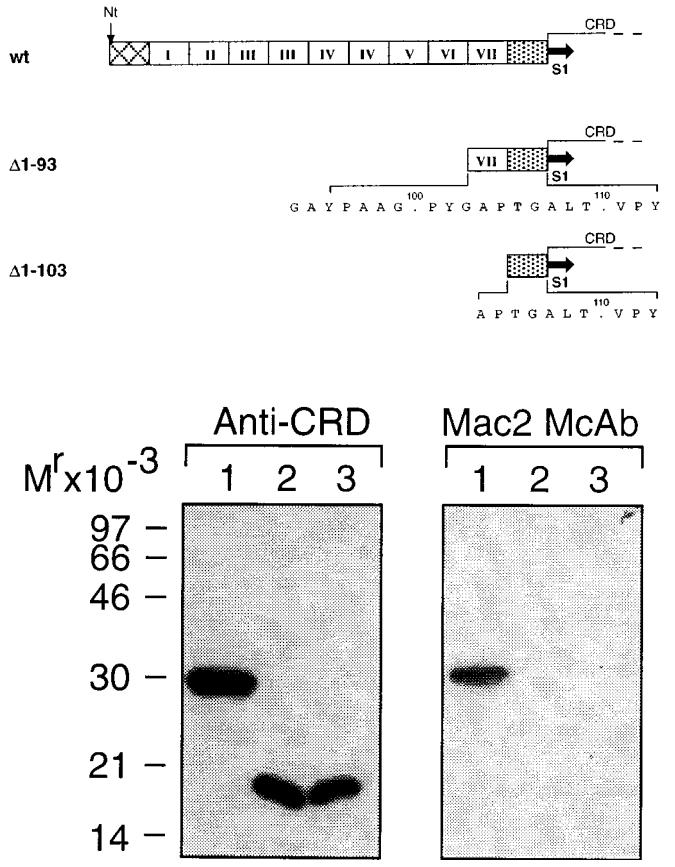

Figure 1. Wild-type and truncated galectin-3. A. Schematic structures. Wild-type (WT) hamster galectin-3 starting from the N-terminus (Nt) with an 18 aminoacid residue segment of average composition (cross-hatched box) followed by nine tandem repeats of proline-rich sequences (open boxes) classified [36] as motifs I–VII and a 12-residue link segment of more average composition (stippled box) that leads into the first β-strand (residues 114–120, thick arrow) of the carbohydrate-recognition domain CRD. The Δ1-93 mutant obtained by deletion of residues 1 to 93. The aminoacid sequence between residues 94 and 113 is shown and comprises most of motif VII and all of the link segment. The Δ1-103 protein obtained from wild-type lectin by collagenase digestion. It lacks N-terminal residues 1 to 103 including all of the repeat sequences and a part of the link segment. B. Western blotting. Samples (approximately 1 μg, respectively tracks 1, 2 and 3) of intact galectin 3, Δ1-93 or Δ1-103 were subjected to reducing SDS-PAGE followed by Western blotting with polyclonal antibodies directed against the CRD or with monoclonal antibody M3/M38 directed against an N-terminal epitope.

have been proposed involving the recruitment and oligomerization of additional lectin subunits by lectin bound to an immobilized substrate such as laminin [8,9].

Several lines of evidence favour the notion that the N-terminal domain plays a major role in self-association and multivalency of galectin 3. Thus, the isolated CRD fragment obtained by collagenase digestion (Fig. 1A) binds to substrates non-cooperatively [8,9]. Secondly, self-association of the recombinant N-terminal fragment of hamster galectin 3 was shown directly by cross-linking [14,19] indicating that similar association might occur between these domains when present in the intact lectin. However, the CRD itself can participate in homophilic interactions producing dimeric and higher forms identified by cross-linking [14,18,20] and might also play some role, in conjunction with interactions between N-terminal domains, in oligomerization of galectin-3. Here, we have used surface plasmon resonance (SPR) to study the kinetics of interactions of hamster galectin-3 with a laminin substrate and the mechanism leading to positive cooperativity in binding to laminin.

Experimental

Murine EHS tumour laminin, bovine fetuin and asialofetuin, and human blood group A-tetrasaccharide were from Sigma, Poole, UK. Endo-β-galactosidase from *Bacteriodes fragilis* and coffee bean α-galactosidase were from Boehringer Mannheim. Recombinant hamster galectin-3 was prepared as described [14]. A CRD fragment (residues 104 to 245) of hamster galectin-3 (Fig. 1A) was obtained by digestion of full-length galectin with *Achromobacter iophagus* collagenase followed by affinity chromatography on an asialofetuin Sepharose column [14]. Rabbit polyclonal antibodies against full length hamster galectin-3 or its CRD fragment were as described [14,19]. Monoclonal Mac-2 antibody [21] was purified from the culture supernatants of the M3/M38 hybridoma (ATCC TIB 166, Rockville Md.) and a Fab fragment was prepared as follows: IgG (6–8 mg) purified by affinity chromatography on Protein G-Sepharose (Pierce) was digested with 26 U papain immobilized on Agarose beads (Sigma) in 0.02 mM TRIS/HC1 pH 6.5 overnight at 4°. After filtration the reaction mixture was passed through Protein G-Sepharose once more and the flow-through fraction containing Fab fragments was dialysed against PBS. Peroxidase-labelled second antibodies were from Sigma.

Plasmids and mutagenesis

N-terminal truncation was performed on wild-type cDNA (clone L1) of hamster galectin-3 in PTMN vector [14]. In order to obtain clone Δ1-93 containing residues 94 to 245 of galectin-3 (Fig. 1A) we used primers SB2 5′-TAT GGA TCC TTA GAT CAT GGT TG-3′ and SB3 5′-TAT CCA TGG GAG CCT ATC CTG CTG C-3′ with a BamH1 restriction site and an Nco1 restriction sites coinciding with the ATG start codon. The PCR was performed with Expand T^M High Fidelity PCR system as follows: 1 cycle at 94 °C for 2 minutes, 10 cycles at 94 °C for 20 seconds, 68 °C for 20 seconds and 72 °C for 45 seconds, 20 cycles at 94 °C for 20 seconds, 68 °C for 20 seconds and 72 °C for 2 minutes with extension time increasing 10 seconds/cycle, followed by 1 cycle at 72 °C for 7 minutes. The PCR product was digested with BamH1 and NCo1 and ligated into a PTMN

vector digested with BamH1 and NCo1. After transformation of E. Coli strain BL21 DE3pLys5, plasmids were purified using a Hybaid kit and nucleotide sequences were verified by dideoxy-chain termination reactions using a sequenase kit version 2.0 (US Biochemical Co.).

Recombinant proteins

Expression of recombinant lectins in E. Coli strain BL21 DE3pLys5 was induced by addition of 0.2mM isopropyl thio-β-galactoside for 4–5 hours. Cells were pelleted, washed with cold PBS, resuspended in lysis buffer 1M TRIS pH7.4 - 5mM EDTA - 10mM 2 - mercaptoethanol- 2 μM aprotinin - 100 μM leupeptin -1 μM pepstatin A - 1mM PMSF - 0.02% NaN3 and sonicated. The suspension was centrifuged and clarified lysate was applied to an affinity column of lactose Agarose (Sigma) equilibrated with 50 mM TRIS pH 7.2 - 150 mM NaCl - 1mM EDTA - 2 mM- 2 - mercaptoethanol - 0.02% NaN_3 - 0.1 mM PMSF. Lectin preparations were then eluted with the same buffer containing 150 mM lactose. The proteins at concentrations up to about 200 μmolar were then passed through a 2 × 70 cm Biogel P60 column in the same buffer without 2-mercaptoethanol to remove lactose and peak fractions (1 ml) were pooled, adjusted to 100 μmolar concentrations and stored at 2 °C. Immediately before use in binding experiments, lectin solutions were passed at room temperature through an 0.5 × 25 cm Sephadex Superfine column. Individual peak column fractions (0.5 ml) collected over 1–2 hours were used for binding studies. Purity of the products was assessed by SDS-PAGE and blotting (Fig. 1B). As expected, intact galectin 3 and the truncated derivatives all reacted with anti-CRD antibody but only intact lectin reacted with Mac-2 antibody recognizing N-terminal epitopes.

Binding assays

Interactions of soluble wild-type or mutant galectin-3 with glycoprotein ligands were monitored with a BIAcore™ 2000 instrument (BIAcore, Uppsala, Sweden). Laminin, or other proteins as indicated, was covalently coupled to a Sensor Chip CM5 (BIAcore) using the Amine Coupling kit and following instructions from the manufacturers. In some experiments, partially de-glycosylated laminin was used. Laminin (0.5 mg) in 1 ml of PBS was adjusted to pH 5.8 or pH 6.5 with 1N HCl and treated respectively with endo-β-galactosidase (40 μl, 52 mUnits) or α-galactosidase (40 μl, 0.5 Units) at 37° overnight. A control chip was prepared using BSA. Briefly, laminin or other protein solutions at various concentrations in 10 mM sodium acetate pH 4.5 were coupled through free amino-groups to the carboxymethylated dextran surface of the chip during 3 to 7 minutes, to allow optimal immobilization. Matrix densities were estimated from the approximation that 1000 response units (RU) is equivalent to 1 ng of protein/mm^2 of surface. Two laminin chips differing in matrix densities, estimated to be approximately 1 and 10 ng/mm^2 respectively, were prepared using initial concentrations of 10 or 100 μg/ml of laminin. Different concentrations of galectin samples (30 μl) in PBS - 5 mM EDTA - 0.02% sodium azide were injected across a test or control chip at a flow rate usually of 10 μl/minute using the instrument in multichannel mode. When regeneration of the sensor surface was needed it was carried out with a 30 seconds pulse of 100 mM lactose in the above buffer. Data transformation was prepared with BIAcore 2.1 evaluation software. Association rate (k_{ass}; M^{-1} S^{-1}) was computed from the binding data at optimal concentration using non-linear fitting statistics. The dissociation rate (k_{diss}; S^{-1}) was measured starting 5–10 seconds after the sample was replaced with buffer. The association constant (Ka) was then calculated ($Ka = k_{ass}/k_{diss}$). Equilibrium binding data were analysed by linear curve-fitting of the Scatchard plots using Cricket Graph.

Results

Laminin from the mouse EHS tumour, a high affinity binding substrate for galectin 3 [8,22–24], contains 25–30% by weight of carbohydrate. The major N-glycans are bi- and tri-antennary complex-type structures carrying polylactosamine chains and capped with α1,3-linked galactose residues [25–28]. In order to establish the specificity of interactions of galectin 3 with laminin immobilized on a BIAcore chip, we prepared surfaces derivitized with laminin treated with α-galactosidase to remove the capping α-galactose residues or with endo-β-galactosidase to remove the polylactosamine units. A chip derivitized with BSA was used as an additional control. The α-galactosyl binding plant lectin GS1-B4 [25] produced an increase in the surface plasmon resonance (SPR) response when injected over the chip surface and this decayed slowly after withdrawal of analyte during washout (Fig. 2B). The specific signal obtained during the association period was eliminated after α-galactosidase treatment and only a small and non-specific shift remained, induced by injection of a relatively high concentration of lectin resulting in a change in bulk solution refractive index (Fig. 2B). By contrast the SPR signal induced by tomato lectin was unaffected by α-galactosidase treatment but was reduced to background after treatment with endo-β-galactosidase (Fig. 2C), in keeping with the specificity of this lectin for polylactosamine chains [29]. The maximum amount of lectin binding to intact laminin is expressed as the maximum SPR signal, R_{max}. The relative binding ration R_{max}/molecular weight of lectin was about 6 times greater for tomato lectin compared with GS1-B4, as expected since GS1-B4 binding is limited to the proportion of chains capped with α-galac-

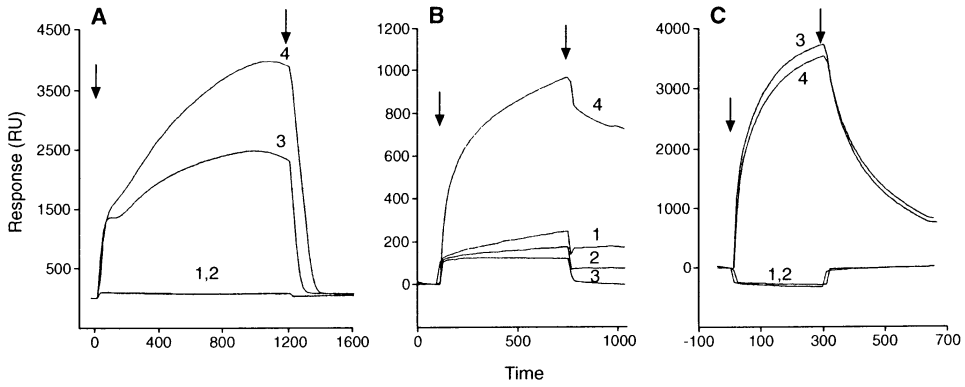

Figure 2. Sensorgrams showing the specificity of lectin binding to intact and partially deglycosylated laminin-surfaces. Samples of solutions of hamster galectin 3 (2.7 μM, A) in PBS-5 mM EDTA, *Griffonia simplicifolia* I-B4 isolectin (10 μM, B) or tomato letin (2.7 μM, C) in PBS - 1mM calcium -1 mM magnesium were passed over surface-bound laminin (curve 4), α-galactosidase-treated laminin (curve 3), endo-β-galactosidase-treated laminin (curve 2) or BSA (curve 1). The arrows indicate the beginning and the end of the injections. RU, response unit.

tose while tomato lectin binds to all polylactosamine chains.

Kinetic analysis

When a solution of galectin 3 was passed over a laminin-derivitised chip, the association phase of the binding reaction was biphasic, including an initial response of about 1500 RU followed by a slower increase of about 2000RU (Fig. 2A). The initial association phase was quantitatively similar during interaction of galectin 3 with α-galactosidase-treated laminin but the second phase response was reduced to about 1000 RU. The dissociation phase of the reactions with intact or de-galactosylated laminin proceeded at similar rates (Fig. 2A). No interaction was detected with chips carrying laminin treated with endo-β-galactosidase or BSA (Fig. 2A). In order to obtain kinetic data on the binding of galectin 3 to laminin, we repeated the above experiment using a range of lectin concentrations up to 2.6 μmolar and a shorter injection time. Identical concentrations of lectin were also passed over the endo-β-galactosidase-treated laminin to correct for any SPR responses due to bulk refractive index changes. In fact, these were found to be very small across the whole range of concentrations examined (Fig. 3A). At sub-μmolar concentrations a single association rate constant 30000 M^{-1} S^{-1} was obtained that together with a dissociation constant of k_{diss} 0.3 S^{-1} produced a calculated Ka of about 1×10^5 M^{-1} (Table 1). At lectin concentrations equal to or above 1 μmolar two apparent association rate constant k_{ass} were obtained: one of 30000 M^{-1} S^{-1} and a second 8000 M^{-1} S^{-1} of lower affinity (Table 1). The dissociation of galectin 3 from both association modes seems to proceed with the same apparent dissociation constant k_{diss} 0.03 S^{-1}, ten-fold slower than the dissociation rate constant estimated at low lectin concentrations. The Ka values derived from these data were 1 and 0.3×10^6 M^{-1} for the higher and lower affinity binding respectively. Binding to the laminin surface of galectin 3 at low (0.65 μM, Fig. 3B) or higher (2.6 μM, not shown) concentrations was effectively inhibited by pre-incubation with lactose or A-active blood group tetrasaccharide, as seen by the progressive reduction in the SPR signal with increasing concentration of competing sugar.

When solutions of the collagenase-derived CRD fragment of hamster galectin 3 (Fig. 1A, Δ1-103) were passed over a laminin surface, the SPR response at each concentration over a broad range was smaller than obtained with equivalent concentrations of the intact lectin (Fig. 3A). After correcting for the difference in molecular weights (Fig. 1B) of intact lectin (32 kDA) and Δ1-103 (18 kDA) using the relationship R_{max}/molecular weight, there is an approximately 5-fold increase in molar binding to laminin of the intact lectin compared with Δ1-103 at the highest concentration (2.6 μM) examined (Fig. 3A). The kinetic constants k_{ass} of 10000 M^{-1} S^{-1} and k_{diss} of 0.2 S^{-1} obtained for Δ1-103 at concentrations up to 10 μM or more in binding to laminin are similar to those obtained for the initial association phase and the single dissociation phase of binding of the intact lectin at low concentrations (0.5 μM or less), leading to similar weak Ka values (Table 1).

Thus, the major effect of the N-terminal domains appears to be to stabilize in some way the lectin once bound to substrate, perhaps by interactions between adjacently bound lectin molecules or by recruitment of additional lectin from free solution. Therefore, we tested the effects of monoclonal antibody M3/38, a rat monoclonal antibody [21] reacting with an N-terminal epitope of hamster galectin 3 (Fig. 1B), on the kinetics of binding to laminin. At relatively high concentrations (1.6 μM) of galectin 3 the SPR response was markedly decreased when the lectin was

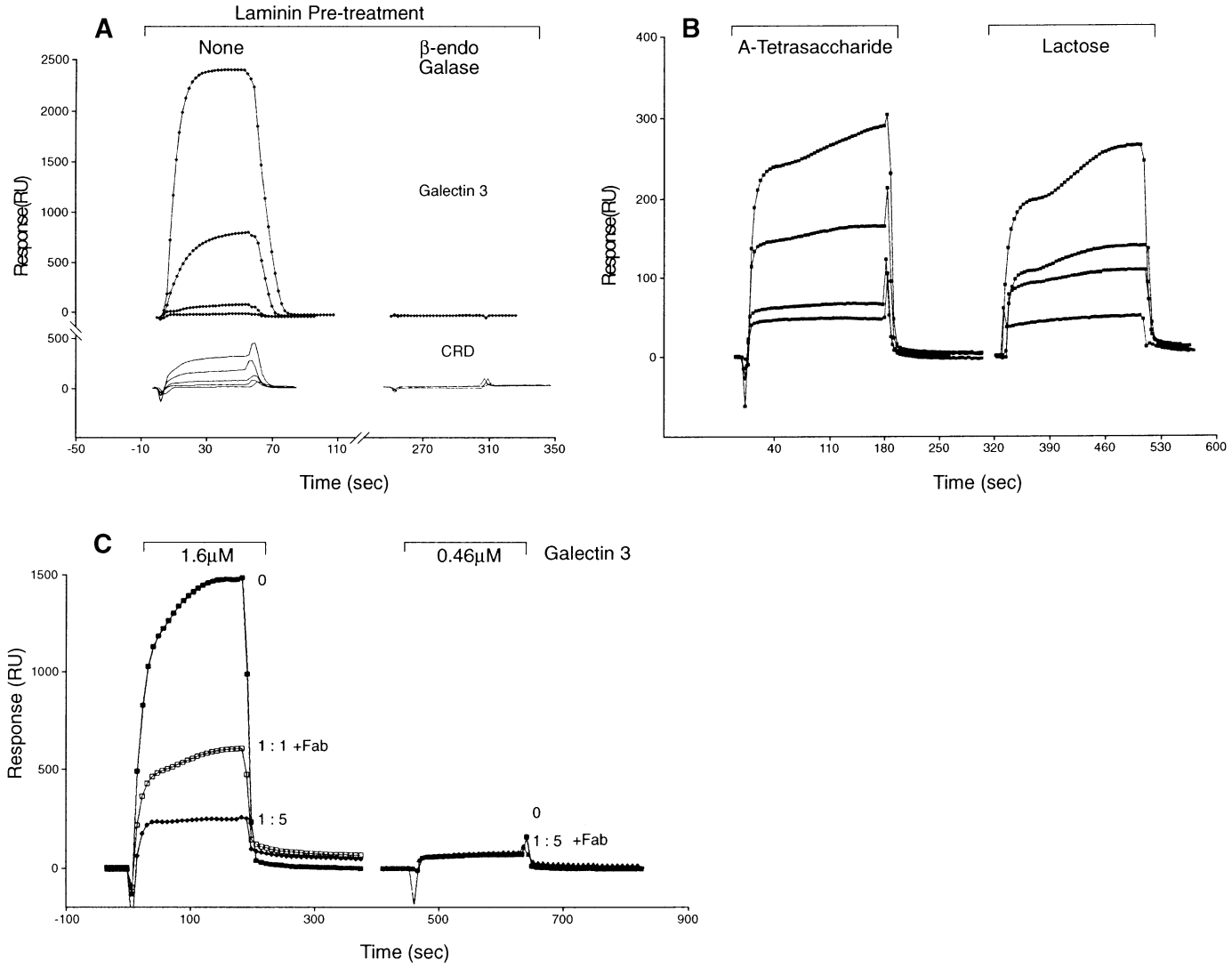

Figure 3. Kinetics of binding of galectin 3 to laminin-coated surfaces. A. Two-fold dilutions of a 2.6 μmolar solution of either intact galectin 3 (upper sensorgrams) or the collagenase-treated CRD fragment Δ1-103 (lower sensorgrams) were injected sequentially over surfaces coated with intact laminin (left-hand sensorgrams) or endo-β-galactosidase-treated laminin (right-hand sensorgrams). Four separate sensorgrams are shown using (from the top) 2.6, 1.3, 0.65 and 0.375 μM solutions. B. Samples of a 0.65 μM galectin 3 solution were incubated at room temperature for 1 hour with either the A-active tetrasaccharide or lactose before injection over a laminin chip. The sensorgrams, from top to bottom, represent four A-tetrasaccharide concentrations (0, 3.7, 14 and 70 μM) or lactose concentrations (0, 85, 170 and 340 μM). C. Samples of galectin 3 solutions (1.6 or 0.46 μM) as indicated were incubated at room temperature for 1 hour with an equimolar or five-fold molar excess of Fab fragments of the M3/38 monoclonal antibody reacting with an N-terminal epitope in the lectin. Control samples lacked Fab fragments. Samples were injected separately over a laminin chip.

mixed with saturating amounts of antibody: inhibition amounted to about 85% after correction for the added weight of the lectin-Fab complex (Fig. 3C). A smaller (25–35%) effect of saturating antibody was obtained using a low (0.46 μM) lectin concentration (Fig. 3C). Interestingly, the monovalent Fab fragment also modulated the binding kinetics of the lectin to laminin, increasing 10 fold the association and dissociation rate constants of the lectin-Fab complex compared with the uncomplexed lectin at comparable concentrations (Table 1). The affinity constant Ka was not changed significantly in presence or absence of Fab (Table 1).

Equilibrium binding measurements

The binding of Δ1-103 to laminin measured over a concentration range up to 20 μM approached saturation (Fig. 4B, inset). A Scatchard plot was linear and gave a Ka 0.3×10^5 M^{-1}, very similar to the value derived from the kinetic constants (Table 1). By contrast, saturation was not ap-

Table 1. Affinity and kinetic data for galectin 3 binding to laminin

	conc μM	$k_a I$ $M^{-1}sec^{-1}$	$k_a II$ $M^{-1}sec^{-1}$	$k_d I$ sec^{-1}	$k_d II$ sec^{-1}	$K_A I$ μM^{-1}	$K_A II$ μM^{-1}
	<0.5	30000 ± 3000		0.3 ± 0.03		0.1 ± 0.05	
wt	>1.0	30000 ± 3000	8000 ± 300	0.03 ± 0.003		>1.0 ± 0.01	0.3 ± 0.05
wt+Fab	1.6	290000 ± 8000		0.5 ± 0.01		0.6 ± 0.04	
Δ1-103	0.4 − 13	10000 ± 3000		0.2 ± 0.06		0.05 ± 0.005	
Δ1-93	>1.0	30000 ± 2000	4000 ± 1000	0.3 ± 0.02	0.002 ± 0.0005	0.1 ± 0.02	>2.0 ± 0.25

proached in binding of intact galectin 3 up to 6 μM concentration (Fig. 4A): higher concentrations could not be examined due to technical difficulties in handling very large SPR responses. A plot of the binding data showed complex sigmoidal relationships and the Scatchard plot gave a concave downwards curve, indicating positive cooperativity of binding (Fig. 4A). Similar results were obtained using chips coated at ten-fold higher matrix density with laminin. As expected, binding of both intact galectin 3 (Fig. 4A) and Δ1-103 (Fig. 4B) was increased, as indicated by the upward shifts of the curves. However, the positions of the maxima along the abscissa in the curves obtained with the intact lectin on the two surfaces were similar, after correction for the different densities of carbohydrate ligands per unit area of substratum, possibly indicating a similar degree of cooperativity [30,31].

Effect of N terminal deletions on binding properties

In view of the very different behaviour in binding to a laminin substratum of intact galectin 3 compared with the CRD fragment Δ1-103, we examined a recombinant galectin 3 truncated in N-terminal sequences and with structural characteristics intermediate between intact lectin and Δ1-103. This derivitive, Δ1-93 (Fig. 1A), lacks the first 93 N-terminal residues including most of the proline-rich repeats but retains part of the last type VII motif, absent in Δ1-103 (Fig. 1A). Interestingly, this protein showed binding properties somewhat different to either intact lectin or Δ1-103 (Fig. 5A). Kinetic analysis of the binding to laminin typically gave values k_{ass} of 30000 M^{-1} S^{-1} and k_{diss} of 0.3 S^{-1} across a broad concentration range (Table 1). These values are similar to intact lectin measured using sub-μmolar concentrations or Δ1-103 at all concentrations examined (Table 1). However, a second mode of interaction was consistently found for Δ1-93 characterised by a very slow off-rate of about 0.002 S^{-1}, one or two orders of magnitude slower than the values obtained for either the intact lectin or Δ1-103, and a relatively high Ka of $>2 \times 10^6$ M^{-1} (Table 1). The slowly dissociating fraction of bound lectin was rapidly displaced by a washout buffer containing 150mM lactose (results not shown), confirming that binding was carbohydrate-dependent. The laminin substrate could be enriched in this component by extending the injection period of a single lectin sample (results not shown) or by repeated injections of Δ1-93 protein (Fig. 5C). By contrast, after consecutive injections of intact lectin the sensorgram fell away to baseline during the final washout with buffer with dissociation rate constant 0.03 S^{-1} (results not shown). The possibility that the slowly-dissociating fraction of bound Δ1-93 protein represented the binding of multimetric aggregates existing in sample solutions was examined by passing the protein through a gel filtration column and analysing individual peak fractions. The sensorgrams obtained were virtually identical for all column fractions and in particular the proportion of the slowly-dissociating species remained constant. Thus, if multimetric aggregates of Δ1-93 protein do form in solution these must rapidly equilibrate to lower forms and are unlikely to participate in multivalent binding to substratum. Further analysis of Δ1-93 binding to laminin revealed very significant positive cooperativity (Fig. 5B), similar to the intact lectin (Fig. 4A).

Discussion

The present data indicate that galectin 3 binds to a laminin-coated surface with reasonably slow on-rates and off-rates and with relatively weak affinity at concentrations below those shown to be required for positive cooperativity in binding to laminin and other substrates [8–10]. At higher concentrations the main difference appears to be in a slower off-rate that, together with on-rates similar to the association rate at low concentration, leads to relatively stronger binding. The overall binding at low or high concentrations depends on interaction between the CRD of at least some lectin molecules with substrate-bound carbohydrates, of which polylactosamine structures seem to be the essential requirement with a smaller but significant contribution from the capping α-galactose residues. In general, the kinetics of galectin 3 binding to laminin appear to be closer to those of plant lectins binding to their carbohydrate ligands than the binding char-

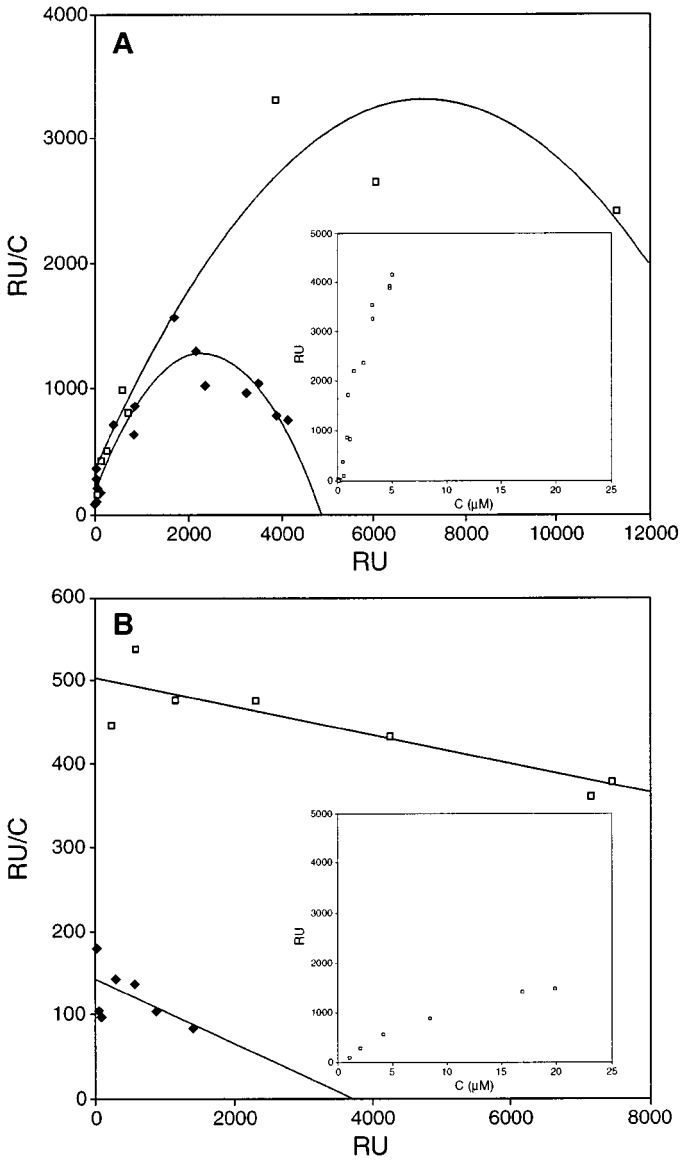

Figure 4. Scatchard plots of galectin 3 binding to immobilized laminin. Increasing concentrations of galectin 3 (A, up to 6 μM) or Δ1-103 (B, up to 20 μM) were injected over a laminin chip (see Fig. 3A). Scatchard plots of the specific binding data, corrected for background binding to an endo-β-galactosidase treated chip (see Fig. 3A), are shown. Two conditions were used to immobilize laminin giving rise to a "high matrix density" chip (10 ng of protein/mm^2 of surface, open symbols) or "low matrix density" chip (1 ng of protein/mm^2 of surface, closed symbols) as described in Methods. Insets: SPR response versus concentration on the low matrix density chip.

acteristics of selectins [32,33] and other [34] C-type mammalian lectins. In the case of the selectins, the on-rates and off-rates are extremely rapid and are thought to be necessary to mediate transient adhesions of leukocytes to cell layers during tethering and rolling. Perhaps the roles of galectin 3 are better accommodated by different binding kinetics, at least when expressed at basal surfaces of polarised epithelia where it appears to contribute to more stable adhesive interactions between cells and a laminin-rich basement membrane [35].

The kinetic parameters obtained for binding of intact galectin 3 to a laminin subtratum fit best with a model [8] in which galectin 3 binds initially through interactions between the CRD with carbohydrate followed by recruitment of additional lectin molecules from free solution. Interactions mediated by N-terminal domains predominate in the latter phase of this process. Each substrate-bound lectin molecule appears to act independently at this stage, since cooperativity was not much influenced by the surface density of bound lectin on the two different laminin substrata (Fig. 4A). We suggest that the slower dissociation rate of lectin bound at concentrations > 1 μM (Table 1) represents dissociation of lectin oligomers. These oligomeric complexes do not form on the laminin surface at sub-μmolar lectin concentrations or after Fab-ligation of N-terminal epitopes and the monomeric bound lectin dissociates with faster kinetics, similar to dissociation of Δ1-103 protein from the substrate.

The ability of the Δ1-93 protein to bind with positive cooperativity to the laminin substratum was suprising, given the presently accepted model for a dominant role of N-terminal domains in cooperative binding of the intact lectin [8,9]. We propose that the high affinity and cooperative binding of Δ1-93 to laminin represents initially monomeric binding followed by stabilization of multimeric aggregates on the substratum. The stability, and probably mechanism of formation, of these aggregates appears to differ from the aggregation of intact galectin 3. On the basis of weak sequence homology with motifs present in other self-aggregating proteins such as synexin VII and elastin, the sequence PGAYPG has been singled out for a potential role in galectin 3 oligomerization [8,16]. This motif is repeated six times in hamster galectin 3 N-terminal domains and one truncated version lacking the first proline residue is also present in Δ1-93 (Fig. 1A). It is possible that this single motif may be sufficient for at least dimerization of the protein but other subunit interaction sites involving the CRD are very likely required for more extensive oligomerization. Similar interactions may also slowly form with substrate-bound intact lectin, but these were not detected kinetically here. Structural analysis and molecular modelling studies of the Δ1-93 protein are in progress to test these proposals, and in particular to identify the nature of the CRD-subunit interaction sites.

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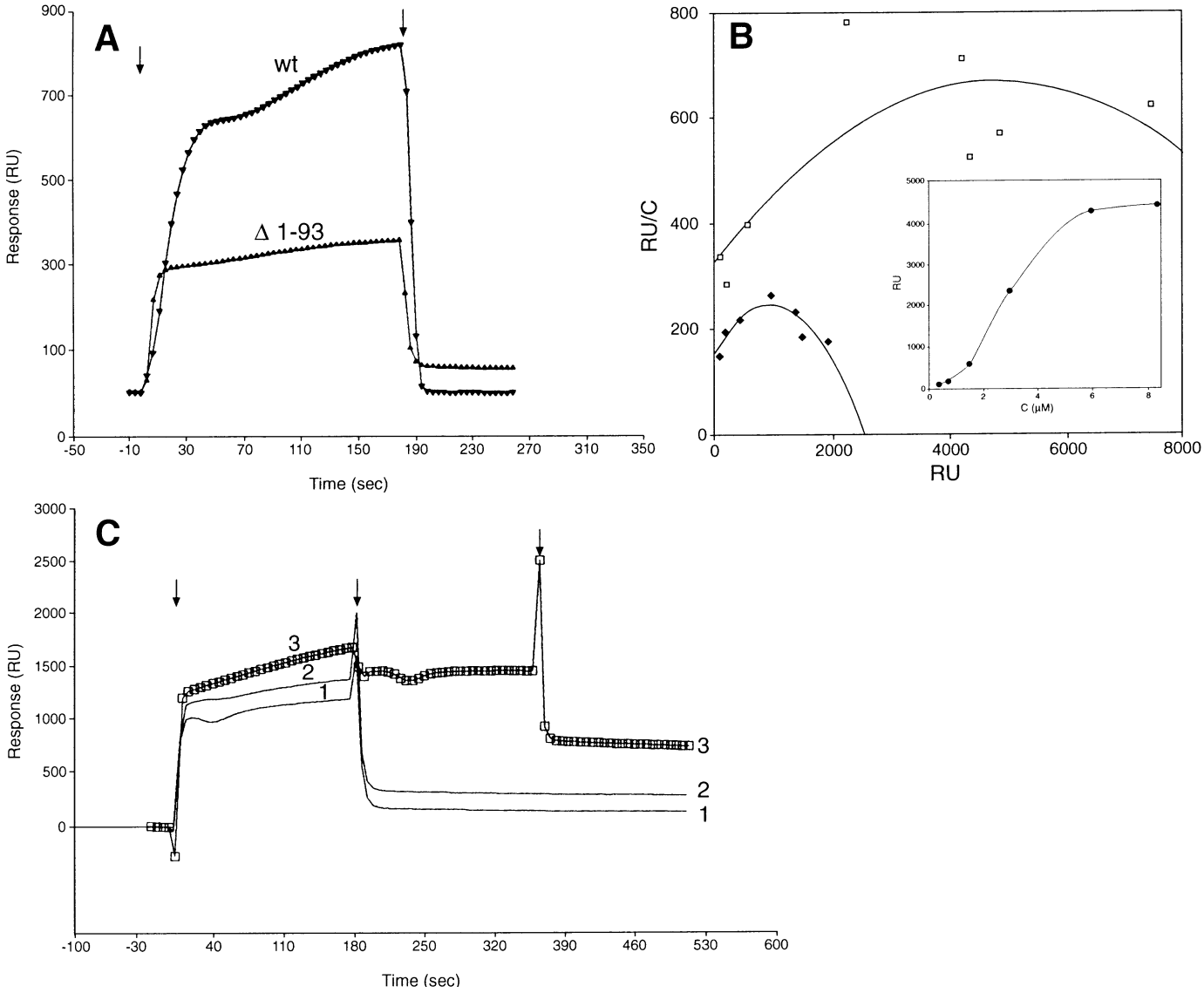

Figure 5. Laminin binding properties of the Δ1-93 mutant of galectin 3 lacking N-terminal domains. A. Sensorgrams of binding of Δ1-93 mutant (2.75 μM) and intact galectin 3 (1 μM) to laminin. Note the biphasic nature of dissociation of Δ1-93, in contrast to the monophasic dissociation of the intact lectin. B. Scatchard plots of Δ1-93 mutant binding to low and high density laminin surfaces as defined in Figure 4. Inset: SPR response versus concentration on the low density chip. C. Samples (3 and 6 μM) of Δ1-93 mutant were passed over a laminin chip separately (curves 1 and 2) or consecutively (curve 3). In the latter case, the times of first and second sample injections and the start of the washout period are indicated by arrows. Note the accummulation of a slowly dissociating fraction during consecutive injections.

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