Selective Recognition of Mannose by the Human Eosinophil Charcot-Leyden Crystal Protein (Galectin-10): A Crystallographic Study at 1.8 Å Resolution[†,‡]

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ABSTRACT: The role(s) of the eosinophil Charcot-Leyden crystal (CLC) protein in eosinophil or basophil function or associated inflammatory processes is yet to be established. Although the CLC protein has been reported to exhibit weak lysophospholipase activity, it shows virtually no sequence homology to any known member of this family of enzymes. The X-ray crystal structure of the CLC protein is very similar to the structure of the galectins, members of a β-galactoside-specific animal lectin family, including a partially conserved galectin carbohydrate recognition domain (CRD). In the absence of any known natural carbohydrate ligand for this protein, the functional role of the CLC protein (galectin-10) has remained speculative. Here we describe structural studies on the carbohydrate binding properties of the CLC protein and report the first structure of a carbohydrate in complex with the protein. Interestingly, the CLC protein demonstrates no affinity for β-galactosides and binds mannose in a manner very different from those of other related galectins that have been shown to bind lactosamine. The partial conservation of residues involved in carbohydrate binding led to significant changes in the topology and chemical nature of the CRD, and has implications for carbohydrate recognition by the CLC protein in vivo and its functional role in the biology of inflammation.

Eosinophils are inflammatory leukocytes that are implicated in the pathogenesis of a large number of allergic diseases and host responses to parasitic infections (*1*). The autocrystallizing Charcot-Leyden crystal (CLC)[1] protein, a major eosinophil constituent comprising about 7−10% of total eosinophil protein, is localized to cytosolic, nuclear, and primary granule subcellular sites (*1*, *2*). Charcot-Leyden crystals were first described in 1853 (*3*) in the spleen and heart blood of a leukemia patient, and in 1872 in the sputum of asthmatics (*4*). Since then, the presence of these characteristic hexagonal bipyramidal crystals in a variety of tissues and body fluids is considered a hallmark of eosinophil-associated inflammation. The CLC protein is a small hydrophobic polypeptide with a molecular mass of 16.5 kDa and 142 amino acids (*5*). Although the CLC protein has been reported to exhibit weak lysophospholipase (LPLase) activity (*6*), it does not show any sequence similarities with the available sequences of prokaryotic or eukaryotic LPLases or other lipolytic enzymes (*2*). This weak enzymatic activity of CLC protein is lost during the process of crystallization, and the resolubilized protein is totally inactive (*7*, *8*).

The crystal structure of the CLC protein, determined at 1.8 Å resolution from crystals identical in morphology to the crystals found in vivo, demonstrated that the protein has a "jellyroll" motif resulting from a tight association between a five-stranded and a six-stranded β-sheet joined by two 3_{10}-helices at the two ends (*8*). The overall structural fold of the CLC protein is similar to those of galectins-1 (*9*), -2 (*10*), and -3 (*11*) and most similar to galectin-7 (*12*), although its amino acid sequence is only moderately identical to those of the members of the galectin superfamily (∼20%). The CLC protein has a carbohydrate recognition domain (CRD) containing 9 of the 13 conserved residues in the CRD of galectins-1, -2, and -7, sharing 6 of 8 residues directly involved in β-galactoside binding. This was suggested by carbohydrate binding studies using solid-phase simple carbohydrates (*8*, *13*). However, recent studies have clearly shown that the CLC protein bound to the cross-linked Sepharose or agarose carbohydrate matrix used in these studies and not to the solid-phase carbohydrates themselves (*14*). In addition to the structural similarities between the CLC protein and other galectins, the intron−exon architecture of the gene encoding the CLC protein is analogous to that of the galectins, with the CRD encoded by a single exon (*15*). In light of the observations described above, the CLC protein was redesignated galectin-10 (*16*). However, the natural carbohydrate ligand of the CLC protein has not yet

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[‡] The atomic coordinates for the Charcot-Leyden crystal protein−mannose complex have been deposited with the Protein Data Bank (file name 1QKQ).

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[1] Abbreviations: CLC, Charcot-Leyden crystal; CRD, carbohydrate recognition domain; LPLase, lysophospholipase; rCLC, recombinant CLC.

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Table 1: Data Collection and Processing Statistics[a]

	lactose	*N*-acetyllactosamine[c]	*N*-acetylglucosamine	mannose	mannose
concentration of carbohydrate (M)	0.3	0.3	1.0	0.1	0.3
maximum resolution (Å)	2.5	1.8	2.0	2.5	1.8
no. of observations	28620	111280	81449	28575	138775
no. of unique reflections	6084	18701	13604	6468	18942
R_{symm} (%)[b]	6.0	8.1	8.6	10.5	7.4
completeness (%) (outermost shell)	81.1 (72.3)	98.1 (94.1)	95.7 (91.8)	86.2 (84.2)	99.3 (97.5)
$I/\sigma I$	8.1	7.3	8.4	6.1	7.8

[a] Space group $P6_522$, with the following unit cell dimensions: $a = 49.6$ Å, $b = 49.6$ Å, and $c = 263.1$ Å. [b] $R_{symm} = \Sigma_h\Sigma_i[|I_i(h) - \langle I(h)\rangle|/\Sigma_h\Sigma_i I_i(h)]$, where I_i is the ith measurement and $\langle I(h)\rangle$ is the weighted mean of all measurements of $I(h)$. [c] Data collected at $\lambda = 0.8373$ Å on station BW7B at EMBL Outstation.

been identified, and the predicted bifunctional role of the CLC protein in eosinophil-mediated inflammatory responses remains speculative.

In this paper, we report the first structure of the CLC protein liganded with a carbohydrate (mannose), providing evidence for the CLC protein being a novel, mannose-binding member of the galectin family. Four different monosaccharide and disaccharide ligands were tested in this study, of which only mannose was found to bind to the CRD of the protein. The manner of mannose binding by the CLC protein is different from that by which other related galectins have been shown to bind lactosamine. The structural basis of mannose binding to the CLC protein was also analyzed by reference to structural differences between the CRDs of the CLC protein and other galectins.

EXPERIMENTAL PROCEDURES

Crystals of the CLC protein were obtained as described previously (*8*). CLC crystals were soaked for 48 h with either 0.3 M lactose, 0.3 M *N*-acetyllactosamine, 1.0 M *N*-acetylglucosamine, or 0.1 or 0.3 M mannose (all from Sigma) in 0.2 M Tris-acetate buffer (pH 7.0). A single CLC crystal was used to collect X-ray diffraction data from each soaking experiment.

Diffraction data were collected at room temperature on a 30 cm MAR research image plate using the Synchrotron Radiation Source (SRS, Daresbury, U.K.) on station PX9.6 ($\lambda = 0.87$ Å). The CLC protein crystal soaked with *N*-acetylglucosamine (1.0 M) diffracted to 2.0 Å resolution, while those soaked with lactose (0.3 M) and mannose (0.1 M) diffracted to 2.5 Å resolution. X-ray diffraction data to 1.8 Å resolution were collected from CLC crystals soaked with *N*-acetyllactosamine (0.3 M) and mannose (0.3 M) at station BW7B [EMBL Outstation, Hamburg, Germany ($\lambda = 0.8373$ Å at room temperature using a 34.5 cm MAR research image plate)]. The cell dimensions of the CLC protein crystals changed less than 1% after soaking in the presence of the carbohydrates. Raw data images were indexed, and corrected for polarization and Lorentz effects using the program DENZO (*17*). All data were scaled and merged using SCALEPACK (*17*), and the intensities were truncated to amplitudes using TRUNCATE (*18*). Data collection and processing statistics are listed in Table 1.

All crystallographic refinement was carried out using the program X-PLOR, version 3.851 (*19*). Phases for structure refinement of the different protein−carbohydrate complexes were obtained from the structure of the free CLC protein (*8*) as a starting model. The free CLC protein structure contains 77 water molecules. Four of these water molecules are located in the CRD and were removed from the starting model prior to refinement, to provide independent unbiased electron density for this region. The rest of the solvent molecules were used in refinement and carefully analyzed using the $2F_o - F_c$ electron density maps. For each complex, the starting model without the carbohydrate was refined against the experimental data in the full range of the resolution that was measured. In the case of the CLC protein−mannose complex, a model of mannose was obtained from the crystal structure of porcine lipase (PDB file name 1ETH) (*20*). The carbohydrate was fitted into the $F_o - F_c$ difference electron density map contoured at 3.0σ using the program O (*21*) (Figure 1B). This CLC protein−carbohydrate model was subjected to further rounds of positional, simulated annealing and temperature factor refinement. The final refinement statistics for the complexes are presented in Table 2.

RESULTS

Identification of Carbohydrate Binding. In all cases (CLC protein−lactose, *N*-acetyllactosamine, and *N*-acetylglucosamine complexes) except for the CLC protein−mannose complex, the $2F_o - F_c$ electron density at the CRD after refinement could easily be identified as belonging to the four water molecules known to be present in the free CLC protein structure (Figure 1A). In the 2.5 Å resolution data set initially collected for the CLC protein−mannose complex, the electron density in the $2F_o - F_c$ maps indicated the presence of a mannose molecule bound to the CLC protein CRD. This was further corroborated by the subsequent determination of the CLC protein−mannose complex structure at 1.8 Å resolution (Figure 1C). The quality of electron density corresponding to terminal mannose oxygen atoms O3 and O4, coupled with a high average *B*-factor of 63.1 Å^2 (occupancy of 1.0) for the carbohydrate, suggested that the protein did not bind mannose with high affinity. Setting the occupancy parameter for the carbohydrate to 0.8 gave a good estimate of the average *B*-factor of 47.9 Å^2 for mannose (*B*-factor ranging from 17.0 to 37.0 Å^2 for the surrounding residues of the mannose in the CRD and an overall *B*-factor for the protein of 29.1 Å^2).

Mode of Binding of Mannose to the CLC Protein. The binding of mannose to the CLC protein involves residues from the concave face of a β-sheet involving β-strands S4, S5, S6a, and S6b and the loop between the latter two (Figure 2). No significant conformational change in the CRD was found in the CLC protein upon mannose binding. Table 3 lists the hydrogen bond and van der Waals contacts between mannose and the CLC protein. The bound mannose is in

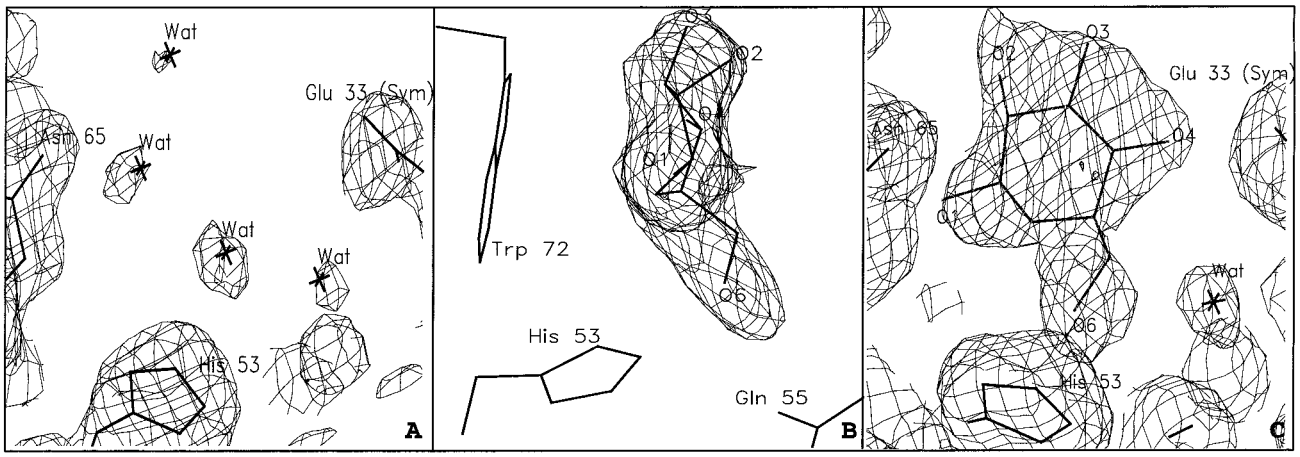

FIGURE 1: Displacement of water upon mannose binding. (A) A portion of the $2F_o - F_c$ SigmaA weighted electron density map (contoured at 1.0σ) in the CRD of the CLC protein in which carbohydrate was not seen to bind can be identified as water molecules. (B) View of a $F_o - F_c$ difference electron density map (contoured at 3.0σ) into which a model of mannose was fitted prior to refinement. (C) A $2F_o - F_c$ SigmaA weighted electron density map (contoured at 1.0σ) of mannose bound to protein (orthogonal view with reference to panel B). The figure was prepared using SETOR (*45*).

Table 2: Structure Refinement Statistics

	lactose	*N*-acetyllactosamine	*N*-acetylglucosamine	mannose (0.1 M)	mannose (0.3 M)
resolution (Å)	40−2.5	30−1.8	40−2.0	99−2.5	40−1.8
R_{cryst} (%)[a]	16.0	21.5	19.6	17.3	20.6
R_{free} (%)[b]	22.5	23.4	24.7	25.6	23.8
no. of reflections[c]	5476	17766	12924	5822	17995
deviations from ideality (rms)					
bond lengths (Å)	0.011	0.009	0.010	0.006	0.007
bond angles (deg)	1.619	1.713	1.561	1.275	1.323
dihedrals (deg)	29.8	30.1	30.0	29.3	30.0
impropers (deg)	1.3	1.3	1.0	0.8	0.9

[a] $R_{cryst} = \Sigma_h|F_o - F_c|/\Sigma_h F_o$, where F_o and F_c are the observed and calculated structure factor amplitudes of reflection *h*, respectively. [b] R_{free} is equal to R_{cryst} for a randomly selected 5 or 10% subset of reflections, not used in the refinement (*43*). [c] Number of reflections in the working set (90 or 95% of unique reflections, such that the remaining test set of 5 or 10% has more than 500 reflections used for the calculation of R_{free}).

the β-configuration (Figure 3). An unusual feature of the interaction is that the carbohydrate adopts a half-chair conformation in the complex and is oriented such that atom O6 of the carbohydrate is buried inside the CRD within hydrogen bonding distance of Nϵ2 of His53, forming an additional hydrogen bond with a water molecule (Wat146). The second unusual feature is that the mannose O1 makes a hydrogen bond with the side chain of Asn65, while O2 forms a hydrogen bond with Gln75 (Figure 3). As in the case of galectin-1 (*9*), galectin-2 (*10*), and galectin-7 (*12*), the glycan ring is stacked against a tryptophan (Trp72 in the CLC protein) (Figure 3, inset 2). A fourth hydrogen bond between Oϵ1 of Glu33 from a symmetry-related molecule and mannose O4 provides further binding interactions (Figure 3, inset 1).

A comparison of the CLC protein−mannose complex with other monosaccharide− and disaccharide−galectin complexes for which structural information is available illustrates striking differences in the mode of binding in the CLC protein−mannose complex. When galectin-7 (*12*) and galectin-3 (*11*) form complexes with galactose, atoms O4−O6 of the carbohydrate are involved in hydrogen bond interactions with the protein. In the case of the galectin-1−*N*-acetyllactosamine complex (*9*), the galectin-2−lactose complex (*10*), and the galectin-7−*N*-acetyllactosamine complex (*12*), atom O4 of the galactose moiety hydrogen bonds with an arginine residue from the protein. However, in the case of the CLC protein−mannose complex, both O3 and O4 atoms of mannose point away from the CRD, resulting in no hydrogen bond interactions between this part of the carbohydrate and the protein.

Carbohydrate Binding Site of the CLC Protein. Galectins share a highly conserved carbohydrate binding site with high affinity for lactose and galactose (*22*), formed by one side of the six-stranded β-sheet of the β-sandwich. The X-ray crystallographic structures of other galectins (*9*−*12*) complexed with different carbohydrates and biantennary saccharides have revealed highly conserved interactions in the CRD of these proteins. The X-ray crystal structure of the CLC protein shows that it shares a high degree of structural similarity with the other galectins involving four β-strands on one face of the protein (*8*). In galectins-1 and -2, this site is defined by residues His44, Asn46, Arg48, Asn61, Trp68, Glu71, and Arg73 (*9*, *10*). Equivalent residues in the CLC protein are His53, Gln55, Cys57, Asn65, Trp72, Gln75, and Glu77. A network of van der Waals and hydrogen bond interactions between these key residues in the CLC protein CRD may serve to stabilize the optimal conformation of the site. For example, His53 forms a hydrogen bond with Asp39, Gln75 is involved in water-mediated interactions with Glu77, and Asn65 has polar interactions with Arg115, Ser66, and Gln74. However, the changes of Arg48 to Cys57 and Arg73 to Glu77 on one side of the CRD lead to a difference in the local charge distribution, and result in the opening of the CRD due to the shortening of these side chains (Figure 4). This might partly explain the changed carbohydrate specific-

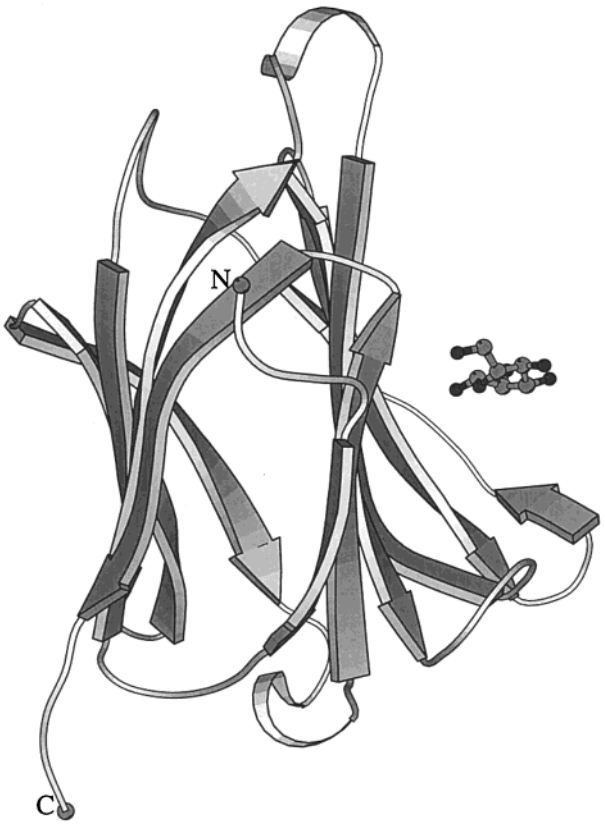

FIGURE 2: Ribbon representation of the CLC protein structure with mannose bound at the CRD.

Table 3: van der Waals and Hydrogen Bond Interactions between the CLC Protein and Mannose

van der Waals contacts[a]			hydrogen bond interactions[b]		
mannose	CLC protein	no. of contacts	donor	acceptor	distance (Å)
C1	Gln75, Asn65	4	His53 Nϵ2	O6	2.59
O1	Gln75, Val63, Asn65	6	Asn65 Nδ2	O1	2.51
C2	Gln75, Asn65, Trp72	4	Gln75 Nϵ2	O2	3.07
O2	Gln75	1	O4	Glu33′ Oϵ2	2.63
C3	Trp72	4	O6	Wat146	2.54
O3	−	0			
C4	Trp72	2			
O4	−	0			
C5	Trp72	2			
O5	His53, Asn65, Trp72	4			
O6	His53	2			

[a] van der Waals contacts are within the maximum allowed values of C−C (4.1 Å), C−N (3.8 Å), C−O (3.7 Å), O−O (3.3 Å), O−N (3.4 Å), and N−N (3.4 Å). [b] Hydrogen bond interactions were calculated using the program HBPLUS (*44*). The residue from a symmetry molecule is denoted with a prime.

ity in the CLC protein. In galectins-1 and -2, these two arginine residues are involved in maintaining a tight association of the protein with the bound saccharide, defining carbohydrate specificity. The change from Glu71 in galectin-1 to Gln75 in the CLC protein does not affect the interactions of this amino acid with the bound carbohydrate. On the contrary, Gln55 and Arg61, which had been previously predicted to provide additional interactions with carbohydrate moieties, are not within hydrogen bonding or van der Waals distances of the mannose moiety. Instead, a hydrogen bond between Glu55 and Glu37, and another

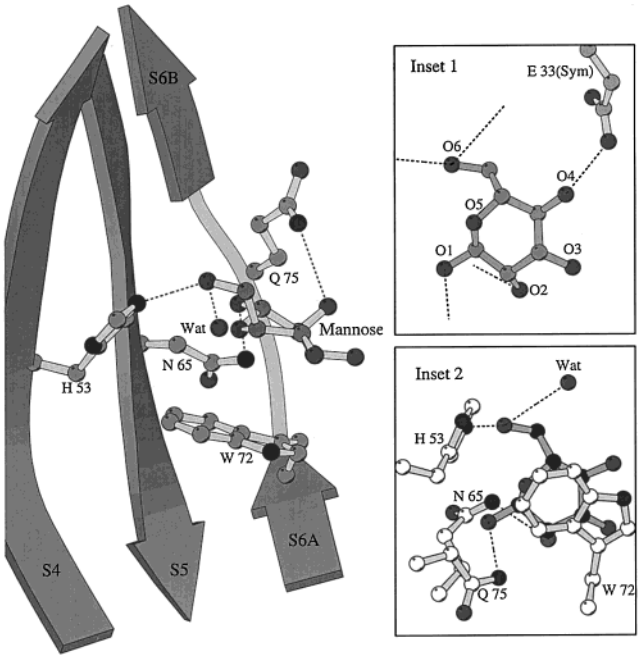

FIGURE 3: Carbohydrate recognition domain of the CLC protein. A detailed view of the CLC protein CRD showing residues His55, Asn65, Trp72, and Gln75 from the protein interacting with bound mannose. Hydrogen bond interactions are represented as dashed lines. Inset 1 is a detailed view of hydrogen bond interactions involving Glu33 from a symmetry molecule and mannose O4. Inset 2 is an orthogonal view of mannose in the CRD depicting stacking interactions against Trp72. The figures were prepared with the program MOLSCRIPT (*46*).

between Arg61 and a water molecule (Wat145), stabilize the charges on these residues.

DISCUSSION

The Charcot-Leyden crystal protein was previously considered to be a member of the LPLase superfamily of proteins despite the lack of apparent sequence or structural homology with any other member of the LPLase family. The CLC protein was reported to exhibit a very *weak* divalent cation-independent LPLase activity with a K_m of 22 μM at 37 °C for lysopalmitoylphosphatidylcholine and a V_{max} of 50 nmol h^{-1} mg^{-1} for the same substrate (*6*, *7*). The crystal structure of the CLC protein in the absence of bound substrate or ligand did not provide direct information about the location of the LPLase active site. Nevertheless, we identified a putative active site some 15 Å away from the CRD on the opposite face of the molecule (*8*). However, the nature and mechanism of LPLase activity for the CLC protein remain to be established. The high degree of structural homology between galectins and the CLC protein has led to its reclassification as a galectin (galectin-10) (*16*). This view is further strengthened by enzymatic assays which have demonstrated that the CLC protein loses LPLase activity irreversibly upon crystallization (*8*, *23*, *24*). The identification of a LPLase similar to pancreatic LPLase in the eosinophil (*25*) has led to speculation that the LPLase activity present in eosinophils may be attributable to this protein instead of the CLC protein. Our results provide further evidence that the CLC protein is more likely to be a lectin and not a LPLase.

We have successfully separated and distinguished the CLC protein from the LPLase activity present in total cell lysates

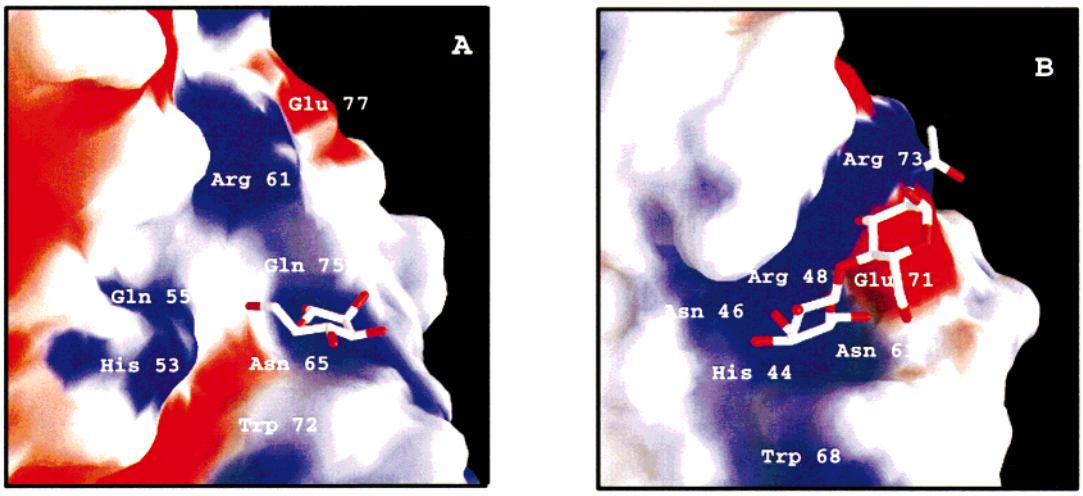

FIGURE 4: Electrostatic surface potential map of the carbohydrate recognition pocket in (A) the CLC protein bound with bound mannose and (B) galectin-1 with bound *N*-acetyllactosamine (*9*) calculated using the program GRASP (*47*), and color-coded on the surface from blue (~5 *kt*/e) to red (~−5 *kt*/e), when *k* is the poisson−Boltzmann constant (joules k^{-1}) at temperature *t* (kelvin) perfection (e). Sequence differences in the CLC protein carbohydrate recognition pocket are reflected in the surface charge distribution and shape of the pocket.

of a human eosinophil cell line (AML14.3D10) using anti-CLC protein antibody affinity columns. Cell lysates completely depleted of the CLC protein (as determined by both a sensitive radioimmunoassay and Western blotting) still possessed LPLase activity comparable to that of the cell lysate prior to CLC protein depletion, an activity that was inhibited by *p*-chloromercuribenzene sulfonate and *n*-ethylmaleimide, two known sulfhydryl group reactive inhibitors of eosinophil LPLase (*6*). In marked contrast, the purified soluble CLC protein from the same cell lysates did not possess any significant LPLase activity, further arguing against the CLC protein being a LPLase (M. P. Savage and S. J. Ackerman, unpublished results).

Initial carbohydrate binding experiments performed with putative carbohydrate ligands had suggested that the CLC protein from lysates of eosinophil-differentiated AMD14.3D10 cells was capable of binding *N*-acetylglucosamine− and lactose−agarose (*8*). Reproducible delayed elution of the CLC protein was observed when cell lysates were passed through an immobilized *N*-acetylglucosamine or lactose affinity column, which could be inhibited by the inclusion of the free carbohydrate in the column or lysis buffer, but not by mannose. The recombinant CLC protein (rCLC protein) expressed in *Escherichia coli* was also reported to bind lactose−agarose and asialofetuin; in this case, binding was inhibited by lactose and fucose, but not by arabinose (*13*). Although similar results could not be observed in CLC protein crystals soaked with *N*-acetylglucosamine or lactose in the present study, our subsequent experiments have shown that the CLC protein binds weakly to Sepharose or agarose matrixes and not to the solid-phase carbohydrates themselves (*14*). On the contrary, even though mannose is observed bound inside the CLC protein CRD in the crystal structure, no delayed elution of the protein could be detected on mannose affinity columns, nor was it possible to detect the CLC protein binding to mannose−Sepharose. These contrasting results lead us to speculate that the carbohydrate binding activity of the CLC protein might be different in the soluble and crystal forms. Since studies aimed at modeling the *N*-acetylglucosamine carbohydrate inside the CRD of the CLC protein do not indicate any steric constraints, the carbohydrate binding activity of the CLC protein requires further investigation.

The ability of the CLC protein to bind mannose in this study draws attention to the functional role of mannose-binding proteins in general. These proteins are C-type lectins which include serum mannose-binding proteins and pulmonary surfactant apoproteins A and D (*26*). Serum mannose-binding proteins play an important role in antibody-independent host defense against pathogens by binding avidly to carbohydrate structures on foreign cell surfaces and inducing opsonization and complement-mediated cell lysis (*27*−*29*). Although the sequence of the CLC protein is not homologous to those of the mannose-binding proteins, it could play a similar role in eosinophil effector or inflammatory responses to multicellular parasites in the body. One of the previously suggested roles for CLC protein, based on its apparent LPLase activity, was in protection from lysophosphatides generated by helminth parasites, and exertion of antiparasitic effects by the hydrolysis of lysophospholipids in the cuticle of larval helminths (*24*). However, in light of the above observations, the role of the CLC protein as an eosinophil lysophospholipase appears to be increasingly untenable.

At the cellular level, the CLC protein is localized to a number of subcellular compartments in eosinophils, including a residual primary granule population, agranular spaces in the cytosol beneath the plasma membrane, and the nucleus (*30*). It has also been colocalized with eosinophil peroxidase in large membrane-bound cytoplasmic vacuoles and other subcellular compartments inside activated macrophages invading tissues. This suggests a role for macrophages in the modulation of eosinophil-rich inflammatory processes (*31*). In freshly isolated, unstimulated human basophils, the CLC protein is primarily present in the histamine-containing granule population. Following activation and subsequent degranulation by the f-met peptide, the CLC protein was shown to relocalize from histamine-containing granules to the plasma membrane, only to resume its basal granule localization by an endocytic and vesicular transport mechanism during basophil recovery (*32*, *33*). These results implicate the CLC protein in the activation, piecemeal

degranulation, and recovery of basophils (*34*).

The CLC protein was also localized to the nucleus and cytoplasm during basophil activation and degranulation (*34*, *35*). While the role of the CLC protein in the nucleus after basophil activation remains unclear, it has been suggested that this protein might bind to the nuclear matrix or chromatin and/or play a role in transcription and RNA processing (*35*). In light of the structural similarity between the CLC protein and the galectin family of proteins, further support for the potential role of the nuclear CLC protein in RNA processing is provided by galectin-3 and galectin-1, which play a role in pre-mRNA splicing (*36*, *37*). The high degree of sequence similarity between the CLC protein and the lectin domain of galectin-3 suggests that it might be involved in eosinophil interactions with the polylactosamine oligosaccharides of extracellular matrix substrates. Galectin-3 binds strongly to laminin (*38*) and induces mast cell histamine secretion by binding to the high-affinity IgE receptor (FcϵRI) (*39*). Galectin-3 has also been implicated as a mitogen capable of stimulating fibroblast cell proliferation, suggesting a role in tissue remodeling (*40*). The recent identification of human ecalectin (*41*), a variant of human galectin-9, as a potent eosinophil chemoattractant causing extravasation of the eosinophil into tissues by activated T-cells, provides yet another possible functional role for the CLC protein in eosinophil inflammatory responses.

GRIFIN, a lens-specific protein, was recently identified as a potential galectin on the basis of the homology of its sequence and those of other galectins, and it does not appear to bind carbohydrates (*42*). However, this protein shows striking similarities to CLC protein in having six out of eight residues implicated in CLC−carbohydrate binding in this report conserved in its putative CRD. These results suggest that both the CLC protein and GRIFIN might form part of a larger family of "galectin-like" proteins with diverse biological functions.

Although we have shown that the crystalline CLC protein binds mannose, the natural glycoconjugate ligand of the protein has yet to be identified. Our results provide a starting point in the search for the biologically relevant ligand(s) of the CLC protein, as well as clues to the functional role(s) in eosinophils and basophils. The unusual features of mannose binding by the CLC protein have set the stage for further investigation of the binding of mannose-specific carbohydrates with this protein. It is also possible that the monosaccharide binding involving the O1 atom points to a role involving the free carbohydrate rather than the sugar as part of an oligosaccharide. We anticipate that further experimental study will provide additional clues about unsuspected function of the CRD and hence for the CLC protein in eosinophil and basophil biology.

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