Electron Microscopy and X-ray Diffraction Studies of *Lotus tetragonolobus* A Isolectin Cross-linked with a Divalent Lewis-x Oligosaccharide, an Oncofetal Antigen*

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Wen Cheng‡, Esther Bullitt‡‡, Lokesh Bhattacharyya¶, C. Fred Brewer‡‡‡, and Lee Makowski¶¶

From the ‡Department of Physics, Boston University, Boston, Massachusetts 02215, the ¶Departments of Molecular Pharmacology, Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461, and the ¶¶Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida 32306-3015

The interactions of lectins with multivalent carbohydrates often leads to the formation of highly ordered cross-linked lattices that are amenable to structural studies. A particularly well ordered, two-dimensional lattice is formed from fucose-specific isolectin A from *Lotus tetragonolobus* cross-linked with difucosyllacto-N-neohexaose, an oligosaccharide possessing the Lewis-determinant, which is an oncofetal antigen. A combination of electron microscopy, x-ray diffraction, simulation of electron micrographs, and molecular model building was used to determine the relative positions of the tetrameric lectin and bivalent carbohydrate within the lattice. X-ray diffraction from unoriented pellets was used to determine the lattice dimensions and analysis of electron micrographs was used to determine the lattice symmetry. Molecular models of the lattice were constructed based on the known structure of the jack bean lectin concanavalin A and the high degree of sequence homology between the two lectins. Using the symmetry and dimensions of the lattice and its appearance in filtered electron micrographs, molecular models were used to determine the orientation of the lectin in the lattice, and to define the range of lectin-oligosaccharide interactions consistent with the structural data. The present study provides the first description of a highly ordered, two-dimensional, cross-linked lattice between a tetravalent lectin and a bivalent carbohydrate.

The carbohydrate moieties of glycoproteins and glycolipids have been shown to be involved in a variety of biological recognition processes including cell-cell and cell-substratum interactions, immunity, apoptosis, and metastasis of tumor cells (1–5). The composition and structures of the oligosaccharides correlate with cell differentiation and transformation (cf. Ref. 6). For example, the expression of oligosaccharides possessing specific Lewis blood group antigenic determinants is developmentally regulated and altered as a result of differentiation and oncogenic transformation (7). Some antigens such as the Lewis-x (Lex)1 antigen are transiently expressed during ontogeny and reappear in tumors, and have hence been termed oncofetal antigens (cf. Ref. 5).

The molecular recognition properties of the oligosaccharide chains of glycoproteins and glycolipids are often characterized in terms of their interactions with lectins (8). A number of mammalian lectins are involved in receptor-mediated endocytosis of glycoproteins (cf. Ref. 2), while others have been implicated in cellular recognition processes including apoptosis (4) and metastasis (9, 10).

The biological signal transduction properties of lectins appear to be due to their ability to bind and cross-link specific glycoprotein and glycolipid receptors on cells. For example, lectin-induced cross-linking of receptors often leads to mitogenic responses in cells (11), in the arrest of bulk transport in ganglion cell axons (12), in the induction of mating reactions in fungi (13), in the molecular sorting of glycoproteins in the secretory pathways of cells (14), and in the apoptosis of activated human T-cells (4). Furthermore, lectin-induced cross-linking of transmembrane glycoproteins leads to changes in their interactions with cytoskeletal proteins and concomitant alterations in the mobility and aggregation of other surface receptors (15, 16).

Studies have shown that lectins undergo two general types of cross-linking interactions with multivalent carbohydrates, designated type 1 and type 2 complexes (17). In a type 1 complex, binding between a divalent lectin and a divalent carbohydrate results in one dimensional cross-linking (e.g. helical). In a type 2 complex, binding between a multivalent lectin and multivalent carbohydrate, where the valency of either the lectin or carbohydrate is greater than 2, results in two-dimensional (planar or tubular) or three-dimensional cross-linking (crystalline). Importantly, type 2 interactions can lead to the formation of homogeneous cross-linked complexes, even in the presence of mixtures of the molecules (cf. Ref. 17). Hence, type 2 interactions are an important source of binding specificity between lectins and glycoconjugate receptors.

In order to understand the molecular basis of these cross-linking interactions, x-ray crystallography has been used to investigate several lectin-carbohydrate cross-linked complexes. These include two type 1 complexes involving dimeric animal lectins cross-linked with divalent oligosaccharides (18, 19). Of type 2 complexes that have been determined, the three-dimensional crystal structures of the tetrameric soybean agglutinin (SBA) cross-linked with four biantennary carbohydrates have

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§ Current address: Dept. of Biophysics, Boston University School of Medicine, Boston, MA 02118-2526.

¶ Current address: Merck Sharpe & Dohme Research Laboratories, West Point, PA 19486.

‡‡ Current address: Department of Physics, Boston University, Boston, Massachusetts 02215.

‡‡‡ Current address: Merck Sharpe & Dohme Research Laboratories, West Point, PA 19486.

** To whom correspondence may be addressed: Depts. of Molecular Pharmacology, Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461.
been reported (20, 21). The x-ray crystallographic images of those structures, refined to 2.4–2.8 Å resolution, indicate that the stability of the lattices are due to protein-carbohydrate interactions, with essentially no direct lectin-lectin interactions. The results also demonstrate that each carbohydrate cross-linked complex with SBA is a unique lattice, in agreement with previous work on type 2 complexes (cf. Ref. 17). The structure of the snowdrop lectin cross-linked with a Man5 oligomannose glycopeptide (22) is another example of a type 2 complex. Interestingly, the glycopeptide exhibits two modes of binding, one involved in cross-linking and one involved in extended side interactions with individual subunits. More recently, the crystal structure of the tetrameric Maclura pomifera agglutinin cross-linked with the T-antigen disaccharide at 2.2 Å resolution has been reported (23). The T-antigen disaccharide functions as a divalent ligand in the complex, even though its binding epitopes are asymmetric.

An exception to the above type 1 and type 2 complexes is the x-ray crystal structure reported for the wheat germ agglutinin cross-linked complex with a glycopeptide isolated from glycoporphin (24). The cross-linked complex consists of divalent-divalent lectin-carbohydrate interactions, in addition to carbohydrate-carbohydrate stabilizing interactions in the complex. It thus resembles a type 1 interaction with secondary homophilic interactions of the carbohydrate ligand.

In the present paper, a structural analysis of the type 2 complex formed by the Fuc-specific tetrameric isolectin A from the seeds of Lotus tetragonolobus (LTL-A) with difucosyllacto-N-neohexaose (Fuc-octa) is described using x-ray diffraction, electron microscopy, image analysis and model building. Our previous work has demonstrated that Fuc-octa binds as a bivalent ligand to LTL-A, and is able to cross-link and precipitate the lectin along with two other analogs of the oligosaccharide (25). The molecular recognition properties of Fuc-octa are important since it is a naturally occurring biantennary fucosyl oligosaccharide possessing the dimeric Leb blood group antigen (Fig. 1). The Leb determinant is an oncotypic antigen since it is transiently expressed during ontogeny and reappears later in a variety of neoplastic diseases (cf. Ref. 5). For example, elevated levels of the Leb antigen are present in patients with lung cancer, and colon and liver adenocarcinomas (5).

LTL-A is a member of the legume family of lectins, which have been widely used to explore the properties of membranes from both normal and transformed cells (26, 27). The x-ray crystal structures of many members of this family have been determined, including concanavalin A (ConA) (28–30), favin (31), pea lectin (32), lentil lectin (33), Lathyrus ochrus isoleucitins I and II (34, 35), Griffonia simplicifolia lectin IV (36), Erythrina corallodendron lectin (37), peanut agglutinin (38), SBA (20), and phytohemagglutinin-L (39). The results demonstrate that this family of proteins have very similar monomeric structures (40), but that they often differ in their dimeric and quaternary structures, particularly among tetrameric lectins such as ConA, the peanut agglutinin, and SBA.

Like most legume lectins, LTL-A is highly homologous to other members of this family of proteins, including ConA, the prototypical Man/Glc-specific lectin from jack bean (41). LTL-A is a tetramer possessing one carbohydrate binding site per subunit of Mr 28,000; hence, it is tetravalent in its carbohydrate binding activity (42). Precipitation of LTL-A by three different fucosyl biantennary oligosaccharides, including Fuc-octa, produces three distinct, highly organized, cross-linked lattices as observed by negative stain electron microscopy (25).

The molecular basis for the stability and uniqueness of these lattices has not been established. The LTL-A/Fuc-octa cross-linked lattice was chosen for analysis because of its highly regular structure as observed by electron microscopy (25). Furthermore, unlike previously described type 2 complexes, which possess three-dimensional lattice structures (20–23), the LTL-A/Fuc-octa complex is the first example of a two-dimensional lattice that is of the type likely to occur on the surface of a cell. The present study thus describes the structure of the lattice in some detail.

**EXPERIMENTAL PROCEDURES**

**Specimen Preparation**—Seeds of *L. tetragonolobus* (syn. *Tetragonolobus purpureas*) were purchased from Schumacher & Co. The native lectin mixture was purified from the crude extract (prepared according to Ref. 42) by affinity chromatography as described previously (43). The major isoelectric, LTL-A, was separated by DEAE-cellulose chromatography (44). Fuc-octa was obtained from BioCarb Chemicals, Lund, Sweden. The concentrations of sugars were measured by the phenol-sulfuric acid method (45, 46). The monosaccharides were obtained from Sigma. LTL-A/Fuc-octa precipitations were performed at 4 °C in a final volume of 0.1 ml by using 0.1 M Tris-HCl buffer, pH 7.2, containing 0.9 M KCl, 1 mM MnCl₂, and 1 mM CaCl₂ as described previously (54).

Concentrations of LTL-A (monomer) and Fuc-octa were 100 μM and 90 μM, respectively.

**X-ray Diffraction**—Pellets of the LTL-A/Fuc-octa precipitate were aspirated into clean, thin-walled glass capillaries (0.5 or 0.7 mm diameter), and sealed with wax. X-ray diffraction patterns from the precipitates were obtained using a rotating anode x-ray source (Rigaku, RU200). The double-mirror camera system (47) was used (CuKα, λ = 1.54 Å) to produce an x-ray beam with dimensions of approximately 0.3 × 0.3 mm at the film (Kodak diagnostic x-ray film). Specimen-to-film distances were 70–130 mm for most experiments, and the exposure times were usually 24–72 h.

**Electron Microscopy**—Precipitates were negatively stained on 300-mesh, carbon-coated Parlodion grids which had been freshly glow discharged, touched to filter paper, floated on a drop of 1% phosphotungstic acid, pH 7.0, and blotted immediately. Samples were observed at 80 kV in a JEOL 1200EX electron microscope (25).

**Electron Micrograph Simulation**—To extract the periodic information and determine the symmetry of the lattice in projection, the electron micrographs were filtered to improve the signal to noise ratio (48). This procedure was implemented by computing the Fourier transform of the image, multiplying the transform by a set of Gaussian functions (one around each of the reciprocal lattice points), and then computing a back Fourier transform to obtain the filtered image.
in the dimer-dimer interactions in the homologous leguminous lectins. Mean phase differences were used as a criterion to evaluate the quality of simulations as detailed by Cabral-Lilly et al. (50).

Molecular Model Building—Using atomic coordinates for ConA (28) obtained from the Brookhaven Protein Data Bank, a fragment of the cross-linked lattice was constructed using the molecular software package FRODO on a Silicon Graphics IRIS workstation. Lattice dimensions were taken from the results of x-ray powder diffraction and the positions of the LTL-A dimers in the lattice from comparison of simulated and actual electron micrographs. Models for the lattice were examined to determine the presence of forbidden contacts and the relative positions of the carbohydrate binding sites.

RESULTS

X-ray Diffraction—X-ray diffraction patterns (Fig. 2) obtained from pelleted samples of the LTL-A/Fuc-octa precipitates consist of a set of concentric reflections falling at the reciprocal spacings listed in Table I. All the observed reflections can be indexed on an orthorhombic lattice with lattice dimensions $85.1 \times 76.1 \times 121.8$ Å. As shown below, the $76.1 \times 121.8$ Å dimensions correspond closely to those observed in electron micrographs of the lattice in projection. The $85.1$ Å dimension corresponds to the thickness of the two-dimensional lattice. Only two “meridional” reflections (second and third order of the $85.1$ Å repeat) are observed. This suggests a partial stacking of the lattices in the pellet. The remainder of the observed reflections correspond to "equatorial" reflections from the two-dimensional lattice. This is typical of diffraction from two-dimensional arrays in which the meridional and equatorial reflections usually dominate the diffraction pattern, with off-equatorial reflections other than the meridional reflections are expected to be weak.

Electron Micrographs—In electron micrographs of negatively stained specimens (e.g. Fig. 3A), the stain-excluding regions appear to be concentrated along two sets of intersecting lines rotated by $62–65^\circ$ relative to one another. Dimensions determined from the micrographs varied slightly, but fell within expected errors of the dimensions obtained in the x-ray powder diffraction experiments. Stain is concentrated in diamond- or triangular-shaped regions between these two linear arrays. Some lattices exhibited substantially greater contrast than others, suggesting the presence of multiple layers. However, the overall distribution of stain-excluding material in the lattices appeared independent of the contrast, suggesting that if lattice stacking did occur, the lattices stack in register. This is consistent with the indexing of the x-ray powder diffraction patterns indicating an orthorhombic lattice and electron micrographs of frozen-fractured pellets.

The power spectrum (square of the Fourier transform) of the electron micrograph in Fig. 3B (enlargement of Fig. 3A) is shown in Fig. 3C. Peaks fall on a lattice with dimensions of approximately $176 \times 1/122$ Å⁻¹. Peaks are observable to approximately $19$ Å resolution in transforms from the most highly ordered specimens. Fig. 3D is a filtered image of the electron micrograph of the lattice in Fig. 3B. In the filtered images of the LTL-A lattices, the stain-excluding material is concentrated at the vertices of the intersecting lines of stain-excluding material noted in the original micrographs. The LTL-A tetramer appears to be centered at these points. The micrograph
in Fig. 3 has mirror lines through these points, corresponding to in-plane two-fold axes in the lattice. The ConA tetramer has 222-point group symmetry and it is likely that the LTL-A tetramer also exhibits this symmetry. One of the molecular two-fold axes probably corresponds to the observed lattice two-fold, greatly limiting the possible models for the molecular structure of the lattice. Modeling of the lattice structure requires the identification of the crystallographic two-fold axis and the location of the two molecular two-fold axes that are non-crystallographic.

Electron Micrograph Simulations—LTL-A is 41.7% identical in sequence to ConA, 39.6% identical to favin, and 40.8% identical to pea lectin (41). The threedimensional structure of the dimers of ConA, favin, and pea lectin are very similar; the root-mean-square difference in α-carbon positions being 0.87 Å for 185 equivalent α-carbons in ConA and pea lectin (32), and 1.4 Å for the 217 common α-carbons of ConA and favin (51). The largest differences are near the ends of the permuted sequence in ConA, and in the vicinity of small insertions and deletions. The difference in dimer structure between ConA and LTL-A should be comparable, making the ConA dimer structure a suitable model for the LTL-A dimer at low resolution.

To generate all possible orientations of the LTL-A tetramer consistent with the observed symmetry of the lattice, models were constructed using the known molecular structure of ConA. Each of the three molecular two-fold axes of the tetramer were aligned with the crystallographic axis in turn. The tetramer was then rotated about that axis in 3° steps, and a simulated micrograph was calculated. The resolution of the simulation was limited to 19 Å to match that of the electron micrographs. Fourier transforms of the simulated micrographs were calculated and a mean phase difference between Fourier transforms of simulated and observed electron micrographs was calculated. Plots of mean phase difference indicated models most consistent with the electron micrographs, but the correspondence between simulated and actual micrographs remained unsatisfactory for even the best models.

The failure of the original modeling to adequately simulate the electron micrographs indicated the need to consider additional modeling parameters. Two that were considered were the possibility of multiple layers and variation of the dimer-dimer contact angle.

To test for the effect of multiple layers, two- and three-layer models were simulated and searched identical to that described above were carried out for a broad range of relative positions of the layers. Correspondence between calculated and observed micrographs became progressively worse for shifts of multiple layers relative to one another. All multi-layer models resulted in poorer correspondence between calculated and observed than for the one-layer models.

Better results were obtained when the relative orientation of the two LTL-A dimers in a tetramer were varied relative to one another. This involved rotating the dimers about the two-fold axis that relates one monomer to the other in each dimer. This variation was suggested by the fact that the structural homology among lectin dimers is very high, but the relative orientations of the dimers relative to one another is somewhat variable. Models were generated by rotating dimers up to 25° relative to one another. The best correspondence between calculated and observed micrographs and the lowest mean phase difference occurred for a relative rotation of the dimers of about 20° in a direction that brought them more nearly parallel to one another. Correspondence of the simulated micrograph with a filtered electron micrograph is demonstrated in Fig. 4.

Molecular Model Building—Construction of a molecular model of the cross-linked lattice made it possible to evaluate potential models for the lattice based on other physical criteria. A viable model for the lattice must have monomers with sugar binding sites separated by an appropriate distance for cross-linking by Fuc-octa. Therefore, the size of the cross-linking carbohydrate provides a constraint on the molecular structure of the lattice. The distance between the two binding epitopes in the oligosaccharide must match the distance between the two carbohydrate-binding sites on adjacent proteins in the lattice.

Three rotamer conformations about the β(1–6) linkage of Fuc-octa are possible, with ω = −60°, 60°, and 180° (ω is the dihedral angle formed by the O-5, C-5, C-6, and O-6 atoms of the core Gal residue). The −60° conformation is considered less energetically favorable by approximately 1–2 kcal/mol by analogy with the solution structure of lacto-N-neohexaose, a biantennary carbohydrate with LacNAc residues linked β(1–6) and β(1–3) to a core Gal (52). As demonstrated below, the tetramer-tetramer contact stabilized by Fuc-octa is at a pseudo-two-fold axis. All three β(1–6) rotamer conformations of Fuc-octa possess pseudo-two-fold axes with respect to the outer Fuc residues which are the binding epitopes for LTL-A (cf. Ref. 53). However, as discussed below, the ω = 60° rotamer of Fuc-octa (Fig. 5) is consistent with the approximate two-fold symmetry of the lattice. In this conformation, the distance between the centers of the two fucose residues is approximately 18 Å. If the two carbohydrate-binding sites are much greater than 18 Å apart, it would be impossible for the oligosaccharide to cross-link adjacent tetramers. If the carbohydrate-binding sites are too close to one another, the oligosaccharide could not participate in an energetically favorable cross-link. Therefore, the distance between the two carbohydrate-binding sites on adjacent tetramers should be approximately 18 Å.

The second criteria for modeling the lattice is to exclude those exhibiting molecular interpenetration (impossibly close contacts) between the protein molecules. Using a molecular model of LTL-A based on a pair of ConA dimers rotated relative to one another, potential models were evaluated on these two criteria. Many models were excluded based on the separation of adjacent binding sites. Forbidden contacts provided less constraint on the possible models as the protein-protein contacts in the lattice are remote from the center of the tetramer. The model with the lowest mean phase difference proved to have acceptable relative positions of the carbohydrate binding sites, and essentially no forbidden contacts.

Molecular Model of the LTL-A Lattice—The candidates for the best model for the cross-linked lattice were selected using the amplitude-weighted mean phase difference and the carbohydrate-binding site distance as criteria. These candidates were then displayed using an optimized gray scale to allow visual comparison of the simulated images with the filtered electron micrographs. The model having the best overall agreement between calculated and observed features is obtained
using the dimers of ConA rotated relative to one another by 20.0° about the x-axis (the two-fold axis relating monomers in a single dimer). The molecular y-axis in ConA corresponds to the crystallographic two-fold axis (mirror plane) in the two-dimensional lattice. The best simulation corresponds to a 12° rotation of the tetramer about this y-axis. The thickness of the two-dimensional array modeled in this way was calculated to be 85 Å. This thickness corresponds with the thickness calculated from the positions of meridional x-ray reflections observed in the powder pattern (Table I). The resulting simulation of an electron micrograph is compared with a filtered image in Fig. 4, and the corresponding molecular model is drawn in Fig. 6.

Fig. 6 shows the relative positions of four lectin tetramers in the lattice. Only α-carbon positions are indicated. In Fig. 6A, the tetramers are seen from the same angle as in the electron micrographs and simulated images. Fig. 6B is an in-plane view of the lattice. In this view, the two tetramers at the center are superimposed. The 12° tilt of the tetramers in the lattice is clear in this image.

**DISCUSSION**

The model of the LTL-A/Fuc-octa lattice presented in Fig. 6 provides substantial information about the way in which LTL-A is cross-linked by Fuc-octa. The full 222-point group symmetry of the lectin is not reflected in the lattice symmetry, as only one molecular two-fold axis is crystallographic. The structure of Fuc-octa is apparently not consistent with a lattice in which the point group symmetry of the LTL-A is optimally crystallographic. Rotation of the LTL-A tetramer by 12° about the y-axis would result in a lattice that would correspond to a single layer of a lattice with C222 symmetry. In this lattice, the
The present study provides insight into the structure of the LTL-A/Fuc-octa cross-linked complex. The results indicate that LTL-A and Fuc-octa form a highly organized two-dimensional cross-linked lattice. The findings demonstrate that the structure and symmetry of the LTL-A/Fuc-octa lattice is dependent on the geometry and symmetry of the binding sites of the lectin and the binding epitopes of the carbohydrate. The present study thus provides evidence for the formation of a highly ordered two-dimensional type 2 cross-linked complex between a tetravalent lectin and a bivalent carbohydrate of the type likely to occur on the surface of a cell. The ability of lectins to form two- and three-dimensional type 2 organized clusters with specific carbohydrate receptors may relate to the biological activities of these proteins. In addition, since Fuc-octa possesses the dimeric Le<sup>a</sup> determinant, which is an oncotic antigen, the cross-linking properties of the oligosaccharide may be important in its biological activities.

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**REFERENCES**

1. Monsigny, M., Roche, A.-C., Kieda, C., Midoux, P., and Obrenovitch, A. (1988) *Biochimia (Paris) 70*, 1633–1649
2. Drickamer, K., and Taylor, M. E. (1993) *Annu. Rev. Cell Biol. 9*, 237–264
3. Barondes, S. H., Cooper, D. N. W., Gitt, M. A., and Leffler, H. (1994) *J. Biol. Chem. 269*, 20995–20998
4. Perillo, N. L., Pace, K. E., Seilhammer, J. J., and Baum, L. G. (1995) *Nature 378*, 736–739
5. Broedhusen, L., and Kuhn, W. (eds) (1997) *Glyкоproteins and Human Disease*, R. G. Landes Co., Austin, TX
6. Kobata, A., and Furukawa, K. (1992) in *Glycoconjugates: Composition, Structure and Function* (Allen, H. J., and Kasiusset, E. C., eds) pp. 33–70, Marcel Dekker, Inc., New York
7. Hakomori, S. (1984) *Annu. Rev. Immunol. 2*, 103–126
8. Allen, H. J., and Kasiusset, E. C. (eds) (1992) *Glycoconjugates: Composition, Structure and Function*, Marcel Dekker, Inc., New York
9. Lotan, R., and Raz, A. (1988) *Ann. N. Y. Acad. Sci. 531*, 385–398
10. Konstantinov, K. N., Robbins, B. A., and Liu, F.-T. (1996) *Am. J. Pathol. 148*, 25–30
11. Nicolson, G. L. (1976) *Biochim. Biophys. Acta 467*, 57–108
12. Edmonds, B. T., and Koenig, E. (1990) *Cell Motil. Cytoskel.* 14, 543–550
13. Niesman, J. J., van der Ende, H., and van Derkate, H. (1991) *J. Biol. Chem. 266*, 15677–15687
14. Chung, K.-N., Walter, P., Aponte, G. W., and Moore, H.-P. (1989) *Science 243*, 192–197
15. Carraway, K. L., and Carraway, C. A. (1989) *Biochim. Biophys. Acta 988*, 147–171
16. Edelman, G. M. (1976) *Science 192*, 218–226
17. Brewer, C. F. (1996) in *Chemtracts Biochem. Mol. Biol.* 6, 165–179
18. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
19. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
20. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
21. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
22. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
23. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
24. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
25. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
26. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
27. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
28. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
29. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
30. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
31. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
32. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
33. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
34. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
35. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
36. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
37. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
38. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
39. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
40. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
41. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
42. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
43. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
44. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
45. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
46. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
47. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
48. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
49. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
50. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
51. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
52. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
53. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
54. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
55. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
56. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
57. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
58. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
59. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
60. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
61. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
62. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
63. Brewer, C. F. (1996) *Chemtracts Biochem. Mol. Biol.* 6, 165–179
Cambillau, C. (1990) Proteins Struct. Funct. Genet. 8, 365–376
35. Bourne, Y., Mazurier, J., Legrand, d., Rouge, P., Montreuil, J., Spik, G., and Cambillau, C. (1994) Structure 2, 209–219
36. Delhaere, I. T. J., Vandomselaer, M., Prasad, L., Quail, J. W., Wilson, K. S., and Dauter, Z. (1993) J. Mol. Biol. 230, 950–965
37. Shaanan, B., Lis, H., and Sharan, N. (1991) Science 254, 862–866
38. Banerjee, R., Mande, S. C., Ganesh, V., Das, K., Dhanaraj, V., Mahanta, S. K., Suguna, K., Suruhi, A., and Vijayan, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 227–231
39. Hamelryck, T. W., Dao-Thi, M.-H., Poortmans, F., Chrispeels, M. J., Wynn, L., and Loris, R. (1996) J. Biol. Chem. 271, 20479–20485
40. Becker, J. W., and Reeke, G. N., Jr. (1989) Trans. Am. Crystallogr. Assoc. 25, 35–50
41. Konami, Y., Yamanoto, K., and Osawa, T. (1990) Fed. Eur. Biochem. Soc. J. 268, 281–286
42. Variv, J., Kalb, A. J., and Katchalski, E. (1967) Nature 215, 886–891
43. Allen, H. J., and Johnson, E. A. Z. (1977) Carbohydr. Res. 58, 253–265
44. Kalb, A. J. (1968) Biochim. Biophys. Acta 168, 532–536
45. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956) Anal. Chem. 28, 350–356
46. Saha, S. K., and Brewer, C. F. (1994) Carbohydr. Res. 254, 157–167
47. Phillips, W. c., and Rayment, I. (1985) Methods Enzymol. 114, 316–329
48. Stewart, M. (1988) J. Electron Microscopy Tech. 9, 301–324
49. Steven, A. C., and Navia, M. A. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 4721–4725
50. Cabral-Lilly, D., Phillips, G. N., Sosinsky, G. E., Melanson, L., Chacko, S., and Cohen, C. (1991) Biophys. J. 59, 805–814
51. Reeke, G. N., Jr., and Becker, J. W. (1986) Curr. Top. Microbiol. Immunol. 139, 35–58
52. Sabesan, S., Duus, J. O., Neira, S., Domaille, P., Kelm, S., Paulson, J. C., and Bock, K. (1992) J. Am. Chem. Soc. 114, 8363–8375
53. Gupta, D., Bhattacharyya, L., Fant, J., Macaluso, F., Sabesan, S., and Brewer, C. F. (1994) Biochemistry 33, 7485–7504
54. Bhattacharyya, L., Haraldsson, M., and Brewer, C. F. (1987) J. Biol. Chem. 262, 1294–1299
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Wen Cheng, Esther Bullitt, Lokesh Bhattacharyya, C. Fred Brewer and Lee Makowski

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