The plasma of the ascidian *Didemnum candidum* possesses lectin activity directed toward galactosyl moieties. We report the purification by affinity chromatography, the physicochemical properties, amino acid composition, and partial N-terminal amino acid sequence of two galactosyl-binding lectins *D. candidum* lectins I and II (DCL-I and DCL-II) from the plasma of this bouchordate species. Both lectins were purified by affinity chromatography (on acid-treated Sepharose 4B and asialofetuin conjugated to Sepharose 4B) to homogeneity as judged by immuneelectrophoresis, size exclusion chromatography on high performance liquid chromatography, and polyacrylamide gel electrophoresis. Isoelectric focusing in polyacrylamide gels revealed that DCL-I focuses as a family of bands at pH 3.8–5.2, while DCL-II focuses at pH 9.2–10.2. Gas chromatography analyses of alditol acetate derivatives indicated that no carbohydrate components are associated with the lectins. Approximate subunit molecular weights estimated by polyacrylamide gel electrophoresis and size exclusion chromatography on high performance liquid chromatography in 6 M guanidine HCl under reducing conditions were 13,400–14,500 for DCL-I and 14,500–15,500 for DCL-II. Native molecular weights estimated by sedimentation equilibrium were 56,600 (DCL-I) and 57,500 (DCL-II), indicating that both species are constituted by four equal-sized subunits. Frictional ratios suggested that both lectins are globular proteins. Using rabbit antisera, the two molecules appeared serologically distinct. The extinction coefficient for DCL-I was $E_{280}^\text{mg} = 2.52 \text{ mg cm}^{-1} \text{ ml}^{-1}$. Circular dichroism analyses of DCL-I suggested 29% $\alpha$-helix and 37% $\beta$-structure in the protein. Excitation/emission fluorescence spectra for DCL-I yielded maximum excitation and emission wavelengths at 288 and 330 nm, respectively. Amino acid compositions of DCL-I and DCL-II differed mainly in the proportions of aspartic and glutamic acids, serine, alanine, cysteine, valine, phenylalanine, and histidine. Amino acid compositions of DCL-I and DCL-II were compared to each other and to immunoglobulins and putative recognition molecules by the parameter $SAQ$. DCL-I exhibited similarities in amino acid composition to lectins from the tunicate *Halocynthia pyriformis*, the lamprey *Petromyzon marinus*, and the horseshoe crab *Carcinoscorpius rotundicauda*, rabbit C-reactive protein, and lamprey and carp immunoglobulin $\mu$ chains. DCL-II showed amino acid composition and similarities with several fish immunoglobulin light chains, immunoglobulin-related molecules isolated from mouse and marmoset T cells, and carp and goldfish immunoglobulin heavy chains. DCL-I N-terminal amino acid sequence showed up to six identities in a stretch of 19 residues with immunoglobulin-related molecules and acute phase proteins (C-reactive protein and serum amyloid P component). DCL-I cross-reacted in enzyme-linked immunosorbent assays with antibodies made against human C-reactive protein.

Although the exact functions of invertebrate lectins are unknown, the findings that lectins of diverse specificities occur both in the serum (1) and on the surface of phagocytic cells (2) suggest that these molecules might play an essential role in self/non-self discrimination. Within the invertebrates, the tunicate group is considered to be evolutionarily the closest group to vertebrates, and, therefore, study of specific recognition molecules in this subphylum should provide clearest evidence for relatedness to vertebrate recognition molecules such as immunoglobulins, complement components, and acute phase proteins. We have previously assessed the distribution of plasma lectins in 10 species of North American tunicates and have found that all species possess detectable lectins and that lectins of distinct specificities occur in the individual species (3). The ascidian *Didemnum candidum* possessed powerful lectin activity directed toward galactosyl moieties. In this paper, we report the purification by affinity chromatography of two galactosyl-binding lectins from the plasma of *D. candidum*. We also describe their physicochemical properties and compare their amino acid compositions and the partial N-terminal amino acid sequence of DCL-I to those of other invertebrate and lower chordate lectins, vertebrate immunoglobulin chains, and other putative recognition molecules, such as acute phase proteins. The sequence and composition comparisons were made in order to investigate the possible relationships of DCL-I and DCL-II to other animal lectins and to the extended family or “superfamily” of immunoglobulin-related recognition molecules (4).

*This investigation was supported in part by Fogarty Institute (National Institutes of Health) Award 1F05TW0515501 to G.R.V. and Grants GM-30672 to J.J.M. and G.R.V. and HL 19491 and HL 26445 to W.W.F. from the United States Public Health Service. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Associated with the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina. To whom correspondence and requests for reprints should be addressed.

1 The abbreviations used are: DCL-I, *Didemnum candidum* lectin I; DCL-II, *Didemnum candidum* lectin II; BF-S, body fluids supernatant; RBC, red blood cells; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; HPLC, high performance liquid chromatography; GdnHCl, guanidine HCl; C-19P, C-reactive protein; SAP, serum amyloid P component; Pth, phenylthiohydantoin; ELISA, enzyme-linked immunosorbent assay; unless noted otherwise, all sugars mentioned in the text, tables, and figures are of the D-configuration.

**Galactosyl-binding Lectins from the Tunicate *Didemnum candidum***

**PURIFICATION AND PHYSICOCHEMICAL CHARACTERIZATION**

(Received for publication, February 6, 1986)

Gerardo R. Vasta, Jeffrey C. Hunt, John J. Marchalonis, and Wayne W. Fish

From the Department of Biochemistry, Medical University of South Carolina, Charleston, South Carolina 29425

THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1986 by The American Society of Biological Chemists, Inc.
Vol. 261, No. 20, Issue of July 15, pp. 9174–9181, 1986
Printed in U.S.A.
Physicochemical Properties of DCL-I and DCL-II—Both species of D. candidum galactosyl-binding lectin exhibited a single sedimenting boundary during sedimentation velocity. Measurements on each species were confined to protein concentrations less than 1 mg/ml, and, under these conditions, a slightly positive dependence of the sedimentation coefficient on protein concentration was observed. Such behavior is suggestive of a tendency of the protein to associate at higher concentrations. This is in keeping with the rather low solubility routinely noted for each of the lectin species. The native molecular weights estimated for each of the lectin species by sedimentation equilibrium, 56,600 and 57,500, suggest that they are of similar molecular weight (Table III). Furthermore, the frictional ratio estimated for each species from its molecular weight and hydrodynamic behavior suggests each is a globular protein.

As shown in Table III, approximate subunit molecular weights estimated from semilog plots of molecular weight versus mobility in SDS-PAGE under reducing conditions (Fig. 6A) were different for DCL-I and DCL-II: 14,500 and 15,500, respectively. As shown in Table III and Fig. 6B, results obtained through gel filtration in 6 M GdnHCl of the reduced and alkylated subunits were slightly lower, but still showed a molecular weight difference of about 1,000 between DCL-I and DCL-II subunits. Thus, both empirical molecular weight estimations of the constituent polypeptide chains of each of the lectin species are consistent with each protein containing four equal-sized polypeptide chains.

As shown in Fig. 7, the far UV circular dichroism spectrum of DCL-I is qualitatively suggestive of a large proportion of \( \beta \)-structure in the protein. Analysis of the circular dichroism data according to the method of Siegel et al. (22) suggests 29\% \( \alpha \)-helix and 37\% \( \beta \)-structure in the protein. The fluorescence spectra of DCL-I (40 \( \mu \)g/ml in Tris-buffered saline) yielded wavelengths of maximum excitation and emission at 288 nm and 330 nm, respectively.

Carbohydrate analysis by gas chromatography of alditol derivatives from DCL-I showed that only traces of mannose and galactose were present in the samples, probably less than 0.01\% each. Such a small amount of galactose could be residual sugar still bound to the combining site of the lectin. A significant glucose peak (1.12\%) observed in the analysis of native DCL-I dropped to 0.09\% for DCL-I samples that were subject to gel filtration in 6 M GdnHCl prior to carbohydrate analysis. This, of course, suggests that the glucose was a contaminant rather than a part of any putative oligosaccharide covalently attached to the DCL-I molecule.

Although size homogeneity was suggested by the behavior of DCL-I and DCL-II on SDS-PAGE or size exclusion chromatography in 6 M GdnHCl, isoelectric focusing in a pH 3–10 range in 6 M urea demonstrated a family of bands focusing at pH from 3.65 to 5.21 for DCL-I (Fig. 8A) and a much less complicated profile for DCL-II (Fig. 8B).

A comparison of the amino acid compositions of DCL-I and DCL-II (Table IV) indicated that the two lectin forms differed mainly in their amounts of aspartic acid, serine, glutamic acid, alanine, cysteine, valine, phenylalanine, and histidine. For DCL-I, the total amount of aspartic and glutamic acid was 32, while histidine, lysine, and arginine totaled 13. For DCL-II, the total amount of aspartic and glutamic acids was 31, while histidine, lysine, and arginine was 16. This suggests that most of DCL-II acid residues are in amidated form since the lectin focuses in the pH range from 9.2 to 10.2. It is noteworthy that methionine was absent in both DCL-I and DCL-II, while there are relatively high proportions of glycine,
alanine, serine, and aspartate in both lectins. The amino acid compositions of DCL-I and DCL-II were compared to each other and to our data base by the $S$Δ$Q$ method (20). Our data base consists of about 250 proteins, including immunoglobulins, myeloma proteins, $\beta$-microglobulin, acute phase proteins, lectins, Thy-1, complement components, and totally unrelated proteins such as $\alpha$- and $\beta$-hemoglobins, HLA antigens, trypsinogen, nerve growth factors, actin, serum albumin and prealbumin, glycoperin A, melanoma antigens, viral glycoproteins, lysozyme, $\alpha$-fetoproteins, $\beta$-endorphin, etc. The parameter $S$Δ$Q$ and related derivatives have been shown to provide a preliminary estimate of relatedness among proteins, even though we emphasize that amino acid sequence data are necessary to confirm the identification. The methods, nevertheless, have been very useful, especially in predicting the conservation of mammalian immunoglobulin $\mu$ chains (23), which was subsequently confirmed by amino acid sequence analysis and for the comparison of a structure of a Thy-1 homolog isolated from a tunicate species with Thy-1 isolated from a vertebrate species (24). Based upon comparisons of unrelated and related proteins carried out by Marchalonis and Weltman (20) and Cornish-Bowden (25), values lower than 80 $S$Δ$Q$ units suggest a high likelihood of relatedness. The frequency of unrelated proteins having values smaller than 100 would be less than 2%. The data of Table V indicate that the galactose-binding protein DCL-I is very similar in amino acid composition to the sialic acid-specific lectin of the tunicate, Halocynthia pyiformis. It also shows similarities to the rabbit C-RP, lamprey serum hemagglutinin and Ig $\mu$ chain, stingray Ig light chains, carp Ig $\mu$ chain, lectin "carcinoscorpin," a lectin from the Indian horseshoe crab Carcinoscorpius rotundicauda, and to heavy chain variable regions. DCL-I differs from DCL-II in 109 $S$Δ$Q$ units, suggesting that they are marginally related. DCL-II shows similarities to carp, goldfish, and trout immunoglobulin light and $\mu$ chains, murine T cell line products, shark and stingray immunoglobulins, and $\gamma$ chain variable regions. Higher $S$Δ$Q$ values suggesting lower degrees of similarity were obtained comparing DCL-I and -II to lectins from Botrylloides, lamprey (Petromyzon marinus) eggs, Limpulus, slug Limax flavus (26), oyster Cossostrea virginica, and the so-called "immune protein" of the silk moth Cecropia.

We performed N-terminal amino acid sequence analysis on purified DCL-I and a comparison of the first 21 amino acids of DCL-I as made to the N-terminal sequence of mammalian and fish immunoglobulins and immunoglobulin variable regions (Table VI). Identities in up to five positions were found between D. candidum lectin DCL-I and various immunoglobulin variable regions. These included the spartic acid ($D$) at position 1, the valine ($V$) at positions 2 and 3, 4, 12, and 19, and the serine ($S$) at position 7. In addition, we compared the first 21 residues of D. candidum lectin DCL-I with N-terminal amino acid sequences of two acute phase proteins, mammalian C-RP (27) and SAP (28), and the lectin from the horseshoe crab Limulus polyphemus (Table VII). Six identities were found between DCL-I and C-RP and three with SAP. No identities occurred with lumulin, but an interesting overlapping was observed between C-RP, SAP, and lumulin in the same stretch where identities with DCL-I, C-RP, and SAP occurred. These data support the results of the amino acid composition analysis (Table V) which suggested that the D. candidum lectin shows a relationship to immunoglobulin variable regions and C-RP. It is interesting that in the short segment which was compared, DCL-I shows highest sequence homology with the same regions of those proteins that exhibit low values of $S$Δ$Q$ with respect to DCL-I.

Finally, we also examined the possible serological cross-reactivities between Didemnum lectins and mammalian C-RP by enzyme-linked immunosorbent assay. Our results (Fig. 9, A and B) showed substantial cross-reactivity of DCL-I with C-RP of human origin. Other lectins tested, such as Halocynthia lectins HPYL-II and III and bovine serum albumin, gave no cross-reaction.

**DISCUSSION**

We have isolated two lectins (DCL-I being the major lectin and DCL-II the minor lectin) from the plasma of the tunicate *D. candidum*. Both lectins are present in the body fluids from individual colonies, as well as pooled fluids. Our previous report on the screening for lectins on 10 species of American tunicates (3) showed that multiple specific lectins are present in all species except *Styela plicata* in which only sialic acid-binding lectins could be detected. Results reported elsewhere (29) suggest that at least three different specific lectins that bind galactose and lactose are present in the plasma of the tunicate *Botrylloides leachi*. We believe that this is the first reported isolation and detailed characterization of a lectin from a protochordate species.

Purification by affinity chromatography on three different galactosyl-containing immunoadsorbents (Sepharose 4B, acid-treated Sepharose 4B, and asialofetuin conjugated to Sepharose 4B) showed that both lectins, although galactosyl-specific, bound differently to the three immunoadsorbents. DCL-I bound to the three immunoadsorbents (the best yield was achieved with acid-treated Sepharose 4B), while DCL-II could only be isolated from the asialofetuin column. Both lectins, however, were effectively eluted from the affinity columns with galactose gradients, with DCL-II requiring a higher galactose concentration for elution than did DCL-I. Isolated lectins DCL-I and DCL-II were homogeneous by SDS-PAGE immunoelectrophoresis and size exclusion HPLC in 6 M GdnHCl of the reduced and alkylated molecules. Based on these results, routine purification of DCL-I and DCL-II was accomplished by the use of two columns: first an acid-treated Sepharose 4B column was used to isolate DCL-I, and, subsequently, DCL-II from the flow-through was isolated on an asialofetuin Sepharose 4B column. This procedure resulted in a recovery of 69% of the total agglutination units processed.

A physicochemical analysis of both *D. candidum* lectins showed that DCL-I and DCL-II are slightly different in both subunit and native molecular weights in that both lectins are composed of four equal-sized subunits of approximately 14,000 and 15,000, respectively. Other properties such as $s_{20W}$, $I_{ave}$, and $v$ (sedimentation coefficient, frictional ratio, and partial specific volume) are similar, although not identical, which suggests that DCL-I and DCL-II are globular proteins of molecular weights about 56,600 and 57,500, respectively. Thus, *D. candidum* lectins are relatively small molecules if compared with lectins isolated from other invertebrate species such as *L. polyphemus* ($M_r = 400,000$) (30), *Tridacna maxima* ($M_r = 470,000$) (31), and *Halocynthia roretzi* ($M_r = 600,000$) (32).

Amino acid compositions of DCL-I and DCL-II are different enough to consider them distinct lectins ($S$Δ$Q$ value is 109 units) and each one might be constituted by isolectins as judged by isoelectric focusing profiles. Although homogeneous in size, both proteins DCL-I and DCL-II exhibit a certain degree of heterogeneity in charge i.e. they focus in gels as a family of multiple bands, DCL-I being more heterogeneous than DCL-II. The amino acid sequence analysis of the DCL-I gave no evidence of heterogeneity in the primary structure of the first N-terminal 21 residues and, of course, heteroge-
neity may occur in other regions of the sequence in order to account for the multiple peaks observed in isoelectric focusing. These isoelectric focusing patterns are reproducible, which suggests that they are not the product of deamination of asparagine or glutamine during the purification procedure. Distinct isoelectins have been demonstrated to be present in invertebrate species such as *Helix pomatia* (garden snail) (33), as well as plants such as *Arachis hypogaea* (peanut) (34).

Circular dichroism analysis indicates that DCL-I contains a high proportion of $\beta$-structure (37%) and also a significant amount of $\alpha$-helix (29%). It resembles the galactosyl-binding lectin from *Tridacna maxima* (31) in the high content of $\beta$-structure (40%); however, *T. maxima* lectin only contains about 10% of $\alpha$-helix. Most lectins from animal and plant sources such as *Helix* (35) and concanavalin A (36) contain a large amount of $\beta$-structure. Exceptions, however, include wheat germ lectin which only contains 10% of $\beta$-structure (37) and limulin III, the lectin purified by Roche and Monsigny (38) from the horseshoe crab, which appears to lack any $\alpha$-helix or $\beta$-structure. Antibodies, on the other hand, contain a high proportion of $\beta$-structure and very little $\alpha$-helix.

DCL-I appears to possess no covalently attached oligosaccharide side chains, and, in this respect, differs from other invertebrate lectins that have been shown to contain large amounts of carbohydrates bound to the protein. These include limulin (99) (24%), *Geodia cyclonum* (40) lectin (9.9%), and *T. maxima* lectin (31) (~7.0%). In this respect, DCL-I resembles the lectins I and II from the sponge *Axinella polypoioidea* (41), which contains only 0.5% carbohydrate, and some plant lectins such as peanut agglutinin, concanavalin A, and garden pea lectins that lack carbohydrate (42).

In addition to the classical immunoglobulin system from vertebrates, several other molecules or families of molecules such as complement components, acute phase proteins (C-RP, SAP, etc.), major histocompatibility complex products, Thy-1 antigens, some humoral bactericidal substances, and humoral or cell-bound invertebrate and lower chordate lectads have been thought to participate in non-self-recognition mechanisms. However, the question of homology among most of these molecules remains open. Although Sir Macfarlane Burnet long ago suggested that invertebrate lectins might be related to the early precursors of immunoglobulins from vertebrates, little subsequent information has been obtained to support this hypothesis. Recent evidence of possible relationships between invertebrate lectads and certain putative recognition molecules from vertebrate include reports on the similarity of combining sites for galactose between myeloma proteins and *Tridacna gigas* lectins (43), the "lectin properties" ascribed to certain acute phase proteins and complement components that were found to precipitate with carbohydrates (44), and the sharing of short stretches of amino acid sequence between putative recognition molecules (21). Work by Kaplan et al. (45) on the amino acid sequence of limulin showed no sequence homology with vertebrate immunoglobulins. However, Robey and Liu (46) found not only that limulin binds phosphocholine as C-RP does, but the two proteins actually possess a short stretch of amino acid sequence with a high percent homology. We have reported (21) that limulin and C-RP cross-react with anti-idiotypic monoclonal antibodies made against the myeloma protein TEPC 15, cross-react with limulin, the sialic acid-binding lectin of the horseshoe crab *L. polyphemus* and C-RP (21). However, the anti-idiotypic monoclonal antibodies did not cross-react with DCL-I. This was not surprising since DCL-I binds galactosyl residues, while the other three molecules, TEPC 15, C-RP, and limulin, bind phosphorylcholine. Polyclonal antibodies made against C-RP cross-react with TEPC 15 and limulin, but also with DCL-I. This suggests that although TEPC 15, C-RP, and limulin might share common determinants related to their binding sites for phosphorylcholine, determinants showed by DCL-I and C-RP are probably located in other regions of the molecule. Moreover, it is relevant to our studies that C-RP has also been reported to bind galactosyl residues and to precipitate with galactans which suggest that C-RP would exhibit more than one specificity (44).

Although very diversified in morphological and ecological
features are probably divergent and far removed from the stem giving origin to chordates, modern tunicates should provide suitable subjects in the search for putative recognition molecules that may exhibit homologies or analogies with members of the vertebrate immunoglobulin family. The results reported here are encouraging in this respect.

Acknowledgements—We thank Melanie Wilson for her assistance in gas chromatography measurements and H. Crow and Steve Martin for expert technical assistance, Maria Teresa Vasta for the preparation of the figures, and Joan Eynon for typing the manuscript.

REFERENCES
1. Vasta, G. R., and Marchalonis, J. J. (1984) in Recognition Proteins, Receptors and Probes: Invertebrates. (Cohen, E., ed) p. 177-191. Alan R. Liss, Inc, New York
2. Vasta, G. R., Cheng, T. C., and Marchalonis, J. J. (1984) Cell. Immunol. 88, 475-488
3. Vasta, G. R., Warr, G. W., and Marchalonis, J. J. (1982) Comp. Biochem. Physiol. 73, 887-900
4. Marchalonis, J. J., Vasta, G. R., Warr, G. W., and Barker, W. C. (1984) Immunol. Today 5, 133-142
5. Vasta, G. R., and Marchalonis, J. J. (1986) J. Biol. Chem. 261, 9182-9186
6. Ersson, B., Asperg, K., and Porath, J. (1973) Biochem. Biophys. Acta 310, 446-452
7. Uhlenbruck, G., Rothe, A., and Parode, G. I. (1968) Z. Immun. Forsch. 136, 73-97
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
9. Babul, J., and Stellwagen, E. (1969) Anal. Biochem. 28, 216-221
10. Laemmli, U. K., and Favre, M. (1973) J. Mol. Biol. 80, 575-599
11. Scheidlegger, J. J. (1956) Int. Arch. Allergy Appl. Immunol 7, 103-110
12. Hunt, J. C., Fish, W. W., and Marchalonis, J. J. (1983) J. Immunol. Meth. 65, 199-205
13. Laurent, T. C., and Killander, J. (1984) J. Chromatogr. 14, 317-330
14. Chervenka, C. H. (1970) Anal. Biochem. 34, 24-29
15. Adler, A. J., Greenfield, N. J., and Fasman, G. D. (1973) Methods Enzymol 27, 675-735
16. Schwabe, C., Anastasi, A., Crow, H., McDonald, J. K., and Barrett, A. J. (1984) Biochem. J. 217, 813-817
17. Alt, J., Heyman, E., and Krisch, K. (1975) Eur. J. Med. 53, 357-369
18. Klapper, D. G., Wilde, C. E., and Capra, J. D. (1978) Anal. Biochem. 85, 126-131
19. Fox, A., Morgan, S. L., Hudson, J. R., Zhu, Z. T., and Lau, P. Y. (1983) J. Chromatogr. 256, 429-438
20. Marchalonis, J. J., and Weltman, J. K. (1971) Comp. Biochem. Physiol. 28B, 609-625
21. Vasta, G. R., Marchalonis, J. J., and Kohler, H. (1984) J. Exp. Med. 159, 1270-1276
22. Siegel, J. B., Steinmetz, W. E., and Long, G. L. (1980) Anal. Biochem. 104, 160-167
23. Marchalonis, J. J. (1972) Nature 236, 84-86
24. Mansour, M. H., Delange, R., and Cooper, E. L. (1985) J. Biol. Chem. 260, 2681-2686
25. Cornish-Bowden, A. (1981) Trends Biochem. Sci. 6, 217-219
26. Miller, R. L., Collawn, J. F., Jr., and Fish, W. W. (1982) J. Biol. Chem. 257, 7574-7580
27. Oliveira, E. B., Gotschlich, E. C., and Liu, T. (1979) J. Biol. Chem. 254, 489-502
28. Osmund, A. P., Friedenban, B., Gewurz, H., Painter, R. H., Hofmann, T., and Shelton, E. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 739-743
29. Schulte, S. F., Ey, P. L., Keough, D. R., and Jenkin, C. R. (1981) Immunology 42, 241-250
30. Marchalonis, J. J., and Edelman, G. M. (1968) J. Mol. Biol. 32, 453-465
31. Baldo, B. A., Sawyer, W. H., Stick, R. V., and Uhlenbruck, G. (1978) Biochem. J. 175, 467-477
32. Yokosawa, H., Sawada, H., Abe, Y., Numakunai, T., and Ishii, S. (1982) Biochem. Biophys. Res. Commun. 107, 451-457
33. Vehslad, P., Hjorth, R., and Lås, T. (1979) Biochem. Biophys. Acta 579, 52-61
34. Miller, R. L. (1983) Anal. Biochem. 131, 438-446
35. Hammarström, S. (1974) Ann. N. Y. Acad. Sci. 234, 183-197
36. Pfleum, M. N., Wang, J. L., and Edelman G. M. (1971) Proc. Natl. Acad. Sci. U. S. A. 68, 2173-2176
37. Thomas, M. W., Walborg, E. F., and Jirgensons, B. (1977) Arch. Biochem. Biophys. 178, 625-630
38. Roche, A-C., and Monsigny, M. (1979) Prog. Clin. Biol. Res. 29, 603-616
39. Finsatd, C. L., Good, R. A., and Litman, G. W. (1974) Trans. N. Y. Acad. Sci. 234, 170-180
40. Müller, W. E. G., Conrad, J., Schroder, C., Zahn, R. K., Kureleć, B., Dreesbach, K., and Uhlenbruck, G. (1983) Eur. J. Biochem. 133, 263-267
41. Breetling, H., and Kabat, E. A. (1976) Biochemistry 15, 3295-3296
42. Sharon, N., and Lis, H. (1972) Science 177, 949-958
43. Eichman, K., Uhlenbruck, G., and Baldo, B. A. (1976) Immunochemistry 13, 1-6
44. Uhlenbruck, G., Solter, J., Janssen, E., and Hahnt, H. (1982) Ann. N. Y. Acad. Sci. 389, 476-479
45. Kaplan, R., Li, S.-L., and Kehoe, M. (1977) Biochemistry 16, 4297-4303
46. Robey, F. A., and Liu, T.-Y. (1981) J. Biol. Chem. 256, 969-975
Galactosyl-binding Lecins from Tunicate D. candidum

MINI-PREP SUPPLEMENT

Galactosyl-binding Lecins from the Tunicate Didemnum candidum. Purification and Phycocyanin Characterization by

G. G. Winter, N. C. Rann, J. M. Martin-Adams and W. M. Fish

Experimental Procedures

Dichroinun body fluids: Twenty-weight percent solutions of dichronin unsedimented whole fluids ranging from 50 to 500 grams were purchased from Gulf Scient Comp., Inc., Panama, Fla. The fluids were centrifuged at 3000 g for 5 min to remove the gross body debris and the cells and the fluids were decanted into 100 mL glass Erlenmeyer flasks were collected on ice. The tr was clamed from de and the dark pigment particles by emulsioning 50 mg of each solution into the supernatant (SF) at a final concentration of 0.24% (w/v) and the SF was stored at -25°C.

Resoactin: Neumeadicans from Viscus bodies (VCB) 120 U/ml and actin were purchased from Genzyme, Boston, Mass. 1:100 dilutions of these two preparations were assayed by use of the SF's from the body fluids. The tr was clamed from de and the dark pigment particles by emulsioning 50 mg of each solution into the supernatant (SF) at a final concentration of 0.24% (w/v) and the SF was stored at -25°C.

Detergents: Ketjen (phosphate) was purchased from Kynshe, Trnmall Park Institute, Bufalo, N.Y. Uniose nonetritol, all other detergents were purchased from Genzyme, Boston, Mass. Ketjen (phosphate) was used in the purification of the SF's from the body fluids.

Desialylation of fetuc: Fetuc was dissolved in 0.02 N HCl, 0.9% NaCl at 10 mg/ml. Hydrolysis was carried out for 1 h at 50°C and the products analyzed as described elsewhere (1). Approximately 70% degradation of fetuc was released from fetuc by this procedure.

Desialylated fetuc was exhaustively dialyzed against 0.8% TCA and thiore stored at 4°C.

Affinity chromatography: Neumeadicans from Viscus bodies (VCB) 120 U/ml and actin were purchased from Genzyme, Boston, Mass. 1:100 dilutions of these two preparations were assayed by use of the SF's from the body fluids. The tr was clamed from de and the dark pigment particles by emulsioning 50 mg of each solution into the supernatant (SF) at a final concentration of 0.24% (w/v) and the SF was stored at -25°C.

Resoactin: Neumeadicans from Viscus bodies (VCB) 120 U/ml and actin were purchased from Genzyme, Boston, Mass. 1:100 dilutions of these two preparations were assayed by use of the SF's from the body fluids. The tr was clamed from de and the dark pigment particles by emulsioning 50 mg of each solution into the supernatant (SF) at a final concentration of 0.24% (w/v) and the SF was stored at -25°C.

Detergents: Ketjen (phosphate) was purchased from Kynshe, Trnmall Park Institute, Bufalo, N.Y. Uniose nonetritol, all other detergents were purchased from Genzyme, Boston, Mass. Ketjen (phosphate) was used in the purification of the SF's from the body fluids.

Desialylation of fetuc: Fetuc was dissolved in 0.02 N HCl, 0.9% NaCl at 10 mg/ml. Hydrolysis was carried out for 1 h at 50°C and the products analyzed as described elsewhere (1). Approximately 70% degradation of fetuc was released from fetuc by this procedure.

Desialylated fetuc was exhaustively dialyzed against 0.8% TCA and thiore stored at 4°C.

Affinity chromatography: Neumeadicans from Viscus bodies (VCB) 120 U/ml and actin were purchased from Genzyme, Boston, Mass. 1:100 dilutions of these two preparations were assayed by use of the SF's from the body fluids. The tr was clamed from de and the dark pigment particles by emulsioning 50 mg of each solution into the supernatant (SF) at a final concentration of 0.24% (w/v) and the SF was stored at -25°C.

Desialylation of fetuc: Fetuc was dissolved in 0.02 N HCl, 0.9% NaCl at 10 mg/ml. Hydrolysis was carried out for 1 h at 50°C and the products analyzed as described elsewhere (1). Approximately 70% degradation of fetuc was released from fetuc by this procedure.

Desialylated fetuc was exhaustively dialyzed against 0.8% TCA and thiore stored at 4°C.

Affinity chromatography: Neumeadicans from Viscus bodies (VCB) 120 U/ml and actin were purchased from Genzyme, Boston, Mass. 1:100 dilutions of these two preparations were assayed by use of the SF's from the body fluids. The tr was clamed from de and the dark pigment particles by emulsioning 50 mg of each solution into the supernatant (SF) at a final concentration of 0.24% (w/v) and the SF was stored at -25°C.

Desialylation of fetuc: Fetuc was dissolved in 0.02 N HCl, 0.9% NaCl at 10 mg/ml. Hydrolysis was carried out for 1 h at 50°C and the products analyzed as described elsewhere (1). Approximately 70% degradation of fetuc was released from fetuc by this procedure.

Desialylated fetuc was exhaustively dialyzed against 0.8% TCA and thiore stored at 4°C.

Affinity chromatography: Neumeadicans from Viscus bodies (VCB) 120 U/ml and actin were purchased from Genzyme, Boston, Mass. 1:100 dilutions of these two preparations were assayed by use of the SF's from the body fluids. The tr was clamed from de and the dark pigment particles by emulsioning 50 mg of each solution into the supernatant (SF) at a final concentration of 0.24% (w/v) and the SF was stored at -25°C.
Table IV: Amino Acid Composition of D. candidum Lectins

| Residue | Total | DCL-I | DCL-U |
|---------|-------|-------|-------|
| Asp     | 18    | 14    | 12    |
| Thr     | 15    | 12    | 9     |
| Ser     | 7     | 5     | 2     |
| Glu     | 6     | 4     | 2     |
| Gly     | 5     | 3     | 2     |
| Ala     | 4     | 3     | 1     |
| Val     | 3     | 2     | 1     |
| Met     | 0     | 0     | 0     |
| Ile     | 5     | 5     | 5     |
| Leu     | 10    | 5     | 5     |
| Tyr     | 2     | 2     | 2     |
| Pro     | 6     | 5     | 3     |
| Cys     | 5     | 5     | 5     |
| Phe     | 2     | 2     | 2     |
| Arg     | 5     | 5     | 5     |

Table V: Closest matches in the comparison between D. candidum lectins DCL-I and DCL-II and the database by S-APl analysis

| Protein     | S-I-Q | Protein     | S-I-Q |
|-------------|-------|-------------|-------|
| Halobacterium HA | 59 | WEH-7 | 44 |
| Rhodobacter L | 77 | C. carpio L | 45 |
| Cainesomespin | 82 | TP-92 lau | 49 |
| Lampry HA | 83 | Dog L | 52 |
| Lampry B | 87 | C. carpio L | 53 |
| Heavy chain variable | 88 | C. carpio B | 54 |
| 7B-BF | 84 | C. carpio UF | 57 |
| C. carpio F | 96 | Dog L | 73 |
| Rabbit CRP | 106 | Mouse IgG | 77 |
| DCL-B | 129 | Lambda variable | 78 |
| C. carpio L | 140 | Mouse IgG | 81 |
| Human B | 146 | Human B | 83 |
| PC/129 mouse kappa | 138 | Mouse IgG | 87 |
| B2.2 lambda 2 | 93 | Rhinobetus L | 93 |
| Rhinobetus L | 96 | Rhinobetus L | 96 |
| Ver B lambda 1 | 98 | Rhinobetus L | 98 |

Table VI: Comparison of N-Terminal Sequences of D. candidum Lectins DCL-I and DCL-II and Representative Proteins

| Residue Number | Number of Identities |
|----------------|---------------------|
| DCL-I | DCL-II |
| 1     | 1     | 1     | 1 |
| 2     | 2     | 2     | 2 |
| 3     | 3     | 3     | 3 |
| 4     | 4     | 4     | 4 |
| 5     | 5     | 5     | 5 |
| 6     | 6     | 6     | 6 |
| 7     | 7     | 7     | 7 |
| 8     | 8     | 8     | 8 |
| 9     | 9     | 9     | 9 |
| 10    | 10    | 10    | 10 |
| 11    | 11    | 11    | 11 |
| 12    | 12    | 12    | 12 |
| 13    | 13    | 13    | 13 |
| 14    | 14    | 14    | 14 |
| 15    | 15    | 15    | 15 |
| 16    | 16    | 16    | 16 |
| 17    | 17    | 17    | 17 |
| 18    | 18    | 18    | 18 |
| 19    | 19    | 19    | 19 |
| 20    | 20    | 20    | 20 |
| 21    | 21    | 21    | 21 |

Figure 1: Purification of Didemnum candidum lectin by affinity chromatography on acid-treated Sepharose column (1.5 x 15 cm) equilibrated with TBS. D. candidum BF-S was applied continuously after washing the column with TBS and elution was accomplished by a gradient of D-gluc (from 0 mM to 50 mM) and 2 nM thiourea were collected.

Figure 2. Purification of Didemnum candidum lectins by affinity chromatography on acid-treated Sepharose column (1.5 x 15 cm) equilibrated with TBS. The purification procedure was the same as in Fig. 1.

Figure 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of Didemnum candidum lectins purified by affinity chromatography. Reduced (0.5 M 2-mercaptoethanol) samples and molecular weight standards were run in 10% polyacrylamide gel using a discontinuous buffer system and stained as described in Materials and Methods. Lane 1: DCL-I (20 llg) and DCL-II (10 llg) lane 2: molecular weight standards (60 llg): phosphorylase b (mol. weight 94,000), albumin (68,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100) and cytochrome c (12,400).

Figure 4. Immunoelectrophoresis of Didemnum candidum BF-S and purified lectins DCL-I and DCL-II samples (20 llg) were run in 1.2% agarose, 0.05% gelatine following the technique of Winter and Williams as described in Materials and Methods and developed with rabbit anti-D. candidum BF-S antiserum (1:100 dilution). Well 1: D. candidum BF-S; Well 2: DCL-I; Well 3: DCL-II.
Galactosyl-binding Lectins from Tunicate D. candidum

Figure 1: Elution profiles of reduced and alkylated DCL-I (approximately 150 ng, upper panel) and DCL-II (approximately 150 ng, lower panel). Proteins were applied to a TSK 3000 SW column and eluted with 6M Gdn HC1 at a flow rate of 1 ml/min. Molecular weight standards were A: chymotrypsinogen A; B: ribonuclease A; C: 62 kDa microglobulin. Absorbance due to iodoacetamide is labeled IA.

Figure 2: Estimation of the molecular weight of D. candidum lectins DCL-I and DCL-II (indicated by the arrows). A: From SDS-PAGE under reducing conditions (0.5% mercaptoethanol). Molecular weight standards are A: egg albumin; B: chymotrypsinogen A; C: trypsin inhibitor; D: BSA. B: From size exclusion chromatography by HPLC of the reduced and carboxymethylated proteins on TSK 3000 gel exclusion. Molecular weight standards are A: human IgG (light); B: ovalbumin; C: chymotrypsinogen A; D: human IgG (heavy); E: fetuin; F: ovalbumin; G: chymotrypsinogen A; H: human IgG (light). For details see under "Materials and Methods".

Figure 6: Isoelectric focusing of D. candidum lectins DCL-I (A) and DCL-II (B). Isoelectric focusing was carried out on a thin layer gel (4% polyacrylamide, 2% carrier ampholytes pH 5-10) at 8 W (mA). The gel was fixed, stained with Coomassie Brilliant Blue R-250 and scanned at 590 nm. For details see under "Materials and Methods".

Figure 7: Circular dichroism spectra of D. candidum lectin DCL-I. A: near UV spectrum. B: far UV spectrum. Measurements were conducted with the protein dissolved in TBS at a concentration of 0.5 to 3.5 mg/ml in 0.5 cm or 10 cm path length cuvettes. The mean residue molecular weight of DCL-I used was 150. For details see under "Materials and Methods".

Figure 8: Cross-reactivity by ELISA of DCL-I with C-reactive protein. A: Binding of sheep antisera anti-human C-RP to human C-RP (DCL-I, DCL-II) and BSA (A). Binding of sheep pre-immune serum is shown in solid symbols. B: Binding of rabbit antisera anti-DCL-I to DCL-I (0) human C-RP (Q) and BSA (L). Binding of rabbit pre-immune serum is shown in solid symbols.