Schwartz, E., Adamany, A. M., & Blumenfeld, O. O. (1980) *Biochim. Biophys. Acta 624*, 531.
Scott, D. M., Harwood, R., Grant, M. E., & Jackson, D. S. (1977) *Connect. Tissue Res. 5*, 17.
Uitto, J., Hoffman, H.-P., & Prockop, D. J. (1976) *Arch. Biochem. Biophys. 173*, 187.

Five Structurally Related Proteins from Affinity-Purified *Maclura pomifera* Lectin†

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ABSTRACT: Affinity-purified *Maclura pomifera* lectin (MPL) elutes from a gel filtration column as a single symmetrical peak with characteristics expected for a single protein of approximately 40 000 daltons. This material can be dissociated into two dissimilar polypeptide chains of approximately 10 000 daltons. Ion-exchange chromatography on DEAE-cellulose resolves affinity-purified MPL into five components. These proteins are structurally related and contain varying proportions of the two polypeptide chains. Two of these tetrameric lectins, each composed solely of one of these chains, display differences in mobility during discontinuous polyacrylamide gel electrophoresis and ion-exchange chromatography, but display no detectable differences in hemagglutination of human erythrocytes and interactions with carbohydrates.

Aqueous extracts of *Maclura pomifera* seeds contain a potent hemagglutinating activity which has been purified by specific adsorption onto insolubilized A+H active hog gastric mucin (Bausch & Poretz, 1977). The affinity-purified hemagglutinin is a lectin of approximately 40 000 g/m which displays a preferential reactivity with α-glycosides of *N*-acetyl-D-galactosamine and D-galactose. Preliminary characterization of the lectin (Bausch & Poretz, 1977) indicates that it is a tetramer composed of two each of two dissimilar polypeptide chains associated by noncovalent bonds. The preparation elutes from an analytical gel filtration column as a single symmetrical peak and focuses in a pH gradient as a narrow band centering at pH 4.75.

We now report that the affinity-purified lectin preparation may be resolved into five structurally related protein components of similar hemagglutinating and carbohydrate binding properties.

Materials and Methods

Affinity purification of the *M. pomifera* lectin (MPL) was accomplished by a procedure described previously (Bausch & Poretz, 1977).

Ion-exchange chromatography of the affinity-purified MPL was performed by using a glass column (0.9 × 140 cm) packed with DEAE-cellulose powder equilibrated with 0.01 M phosphate buffer, pH 7.8. After application of the protein solution, fractionation was accomplished with a linear gradient of NaCl (0–0.4 M) in 0.01 M phosphate buffer, pH 7.8. The optical density of every 5-mL fraction and the electrical conductivity of every third fraction were determined.

Polyacrylamide gel electrophoresis was accomplished by using stacking and separation gel components as described by Davis (1964) except that the gels were cast as slabs rather than cylinders. Electrophoresis in the presence of sodium dodecyl sulfate ($NaDodSO_4$) was performed in 10% polyacrylamide gels with 0.1% $NaDodSO_4$ as reported by Laemmli & Favre (1973).

Hemagglutination and saccharide inhibition of agglutination were accomplished as described earlier (Bausch & Poretz, 1977). Amino acid analysis was performed on 22-h HCl hydrolysates of proteins as described previously (Bausch & Poretz, 1977).

Results

Polyacrylamide gel electrophoresis of affinity-purified MPL yields five closely spaced protein staining bands (Figure 1, lane M). These related proteins may be separated by DEAE-cellulose chromatography with a linear salt gradient. Figure 2 shows a relatively symmetrical elution profile of protein from the ion-exchange column. However, gel electrophoresis of select fractions (numbers 63, 73, 81, 92, and 100) demonstrates resolution of the five protein components (Figure 1, lanes A–E). Separation of the proteins is difficult, and only small quantities of the individual components have been obtained. Most fractions of the eluate consist of two or more of the components. However, judicious selection of individual fractions, which may be pooled, results in a yield of purified components equivalent to up to 30% of the applied protein. Ion-exchange chromatography with DEAE-cellulose columns as short as 40 cm has produced resolution of the proteins

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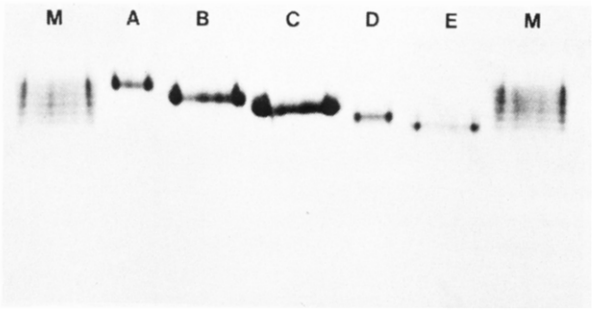

FIGURE 1: Polyacrylamide gel electrophoresis of the proteins of MPL: M, affinity-purified MPL; A, fraction 63; B, fraction 73; C, fraction 81; D, fraction 92; E, fraction 100.

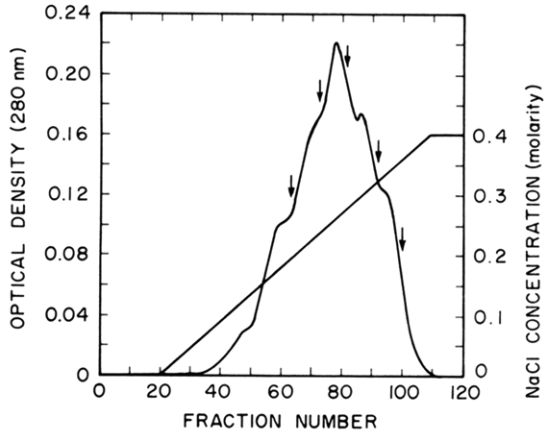

FIGURE 2: DEAE-cellulose ion-exchange chromatography of MPL. Arrows indicate select fractions shown in Figures 1 and 3.

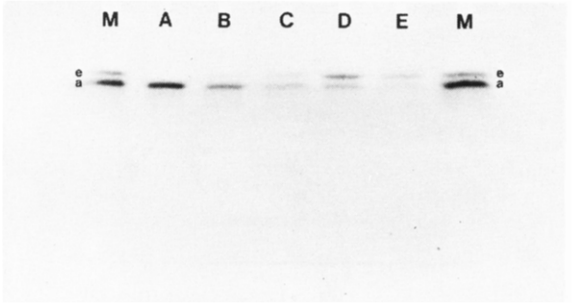

FIGURE 3: Polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$ of the proteins of MPL: M, affinity-purified MPL; A, fraction 63; B, fraction 73; C, fraction 81; D, fraction 92; E, fraction 100.

similar to that obtained with a column of 140 cm in length.

$NaDodSO_4$ gel electrophoresis of the individual fractions isolated following ion-exchange chromatography of MPL (fractions 63, 73, 81, 92, and 100), each containing one of the electrophoretically distinct components, demonstrates (Figure 3) that they consist of differing amounts of two dissociable polypeptide chains. Fraction 63 which contains only component A possesses only the polypeptide displaying the faster electrophoretic mobility ("a"). Fraction 100 which contains only component E has only the polypeptide of slower electrophoretic mobility ("e"). Fractions containing components B–D display decreasing amounts of polypeptide a with proportionately greater amounts of the e polypeptide. These data suggest that the tetrameric proteins A–E may be represented as a_4, a_3e, a_2e_2, ae_3, and e_4, respectively.

However, amino acid analysis of components A and E, each composed solely of subunit a or e, respectively, results in no demonstrable differences in the composistion of these two

Table I: Amino Acid Composition of *Maclura pomifera* Lectins

amino acid	residues per 10 000 g		
	component A	component E	MPL[a]
Lys	5.2	5.3	6.5
His	0.8	0.9	0.6
Arg	3.9	2.9	2.5
Asp	10.0	9.7	9.2
Thr	8.7	8.7	7.4
Ser	7.9	7.9	6.8
Glu	6.8	7.2	7.5
Pro	3.3	4.0	4.1
Gly	15.1	14.7	13.0
Ala	2.4	2.0	2.4
Val	7.3	7.3	7.0
Met			0.5
Ile	5.5	6.3	6.4
Leu	6.1	5.9	5.2
Tyr	4.3	4.4	5.5
Phe	6.2	6.2	5.9
Trp			1.0

[a] Affinity-purified MPL recalculated from the data of Bausch & Poretz (1977).

polypeptides (Table I). The experimental error of ±5% in the analyses, and difficulty in accurately quantitating the minor amino acids, may result in an uncertainty of ±1 residue for the amino acids in greatest quantity, as well as for the minor amino acids, such as arginine, histidine, alanine, and proline. No attempt was made to quantify tryptophan and cysteine. Although analysis was peformed only on 22-h acid hydrolysates of the proteins, amino acid analyses of components A and E prepared at different times provided identical compositions, as reported here. Upon review of the analysis of MPL reported earlier (Bausch & Poretz, 1977), it was realized that the data were reported as residues per 36 515 g rather than per 40 000 g as intended. However, recalculation of the amino acid composition of MPL based upon 10 000 g gives essentially the same composition as do the separated components (Table I).

The similarities exhibited by components A and E are also extended to the ability of these proteins to interact with cells. The minimum concentration of lectin needed to cause complete agglutination of type O human erythrocytes from six individuals averaged 120 and 140 ng/mL components A and E, respectively. Furthermore, the differences in the quantities of lectins needed to agglutinate cells from different individuals did not vary greater than differences in the titrations of cells from the same person. Consistent with these observations, methyl α-D-galactopyranoside, *N*-acetyl-D-galactosamine, and D-galactose inhibited hemagglutination by components A and E to the same degree. Both methyl β-D-galactopyranoside and lactose were unable to cause inhibition of either component. The quantities of each saccharide needed to cause inhibition of agglutination were similar to those reported earlier (Bausch & Poretz, 1977) for the inhibition of hemagglutination by affinity-purified MPL.

Discussion

Affinity-purified MPL may be resolved into five electrophoretically distinct components. Since the original mixture elutes from a gel filtration column as a single symmetrical peak (Bausch & Poretz, 1977), it is presumed all components of the mixture have similar molecular weights. The relationship of the components is evident in that they are composed of either one or both of two polypeptide chains found in the original mixture. Thus, component A, which is apparently the most electropositive protein (being retained the poorest on the DEAE-cellulose column), is composed of four a chains.

Component E, the most electronegative protein, contains four e polypeptide chains. The three remaining components of intermediate electronegativity are composed of differing proportions of the a and e chains.

Surprisingly, the two polypeptide chains have virtually identical amino acid compositions. In light of this, the differences observed for the behavior of the tetramer of each chain (components A and E) during ion-exchange chromatography or the migration of the monomeric units during $NaDodSO_4$ gel electrophoresis may be due to only minor changes involving amino acid substitutions and/or post-translational modifications. Though differences in the carbohydrate moieties of the polypeptides of MPL may cause the differences observed in the properties of the a and e chains, similar to the observations of Carter & Etzler (1975) reported for the A and B subunits of *Dolichos biflorus*, the virtual absence of neutral carbohydrate in MPL (Bausch & Poretz, 1977), which is consistent with the nonreactivity of the lectin with an antibody capable of recognizing the core structure of asparagine-linked oligosaccharides of glycoproteins (Kaladas & Poretz, 1980), makes this possibility less likely than other modifications such as amidation, N-terminal amino group blockage, or polypeptide chain cleavage. It is also noteworthy that Noel et al. (1979) observed differential mobility during electrophoresis in $NaDodSO_4$ of the histidine-transport protein produced by a mutant and the wild-type strains of *Salmonella typhimurium*, which differ only by the nature of one amino acyl residue. These results led the authors to conclude that minor differences other than in molecular weight of polypeptides may affect their electrophoretic mobility during $NaDodSO_4$–acrylamide gel electrophoresis. Amino acid analysis of affinity-purified MPL (Bausch & Poretz, 1977) noted only two residues of methionine per tetrameric molecular weight (40 000). However, CNBr treatment (unpublished experiments) causes cleavage of both $NaDodSO_4$-dissociable subunits of MPL. These results suggest that the small amount of methionine reported originally was low, and a value of at least four residues of methionine per 40 000 g would be more probable. Furthermore, we have found that each component of the mixture acts as a discrete, stable entity. Incubation at 37 °C in phosphate-buffered saline, pH 6.8, for 18 h of a mixture containing 100 μg/mL each of purified components A and E results in no detectable products of rearrangement (that is, components B–D). Polyacrylamide gel electrophoresis shows only components A and E. The lack of subunit exchange under these conditions suggests that the five proteins did not arise artifactually during extraction and affinity adsorption of the lectin.

Consistent with the small differences in the structure of the a and e polypeptide chains, components A and E show no observable differences in the ability of these tetrameric proteins to react with the glycoconjugates of human erythrocytes and simple saccharides.

Results similar to these describing variants for the lectins from wheat germ (Rice & Etzler, 1975), *Phaseolus vulgaris* (Miller et al, 1975), and *Bandeira simplicifolia* (Murphy & Goldstein, 1979) have been reported in the literature. As with these lectins, which have been described as consisting of isolectins, and with the similarity of the characteristics of the a and e polypeptide chains of MPL, we suggest that MPL contains structurally related proteins capable of specifically binding to insolubilized hog gastric mucin and which show similar carbohydrate binding properties. The five proteins of MPL may be represented as a_4, a_3e, a_2e_2, ae_3, and e_4. Such a configuration has been suggested for the isolectins of *Phaseolus vulgaris* (Miller et al., 1975) and contrasts sharply with that of the five lectins isolated from pokeweed, which exhibit identical carbohydrate binding properties (Basham & Waxdal, 1977; Yokoyama et al., 1978) yet have different and, in part, seemingly unrelated physiochemical and structural characteristics (Waxdal, 1974).

References

Basham, T. Y., & Waxdal, M. J. (1977) *J. Immunol. 118*, 863.

Bausch, J. N., & Poretz, R. D. (1977) *Biochemistry 16*, 5790.

Carter, W. G., & Etzler, M. E. (1975) *J. Biol. Chem. 250*, 2756.

Davis, B. J. (1964) *Ann. N.Y. Acad. Sci. 121*, 404.

Kaladas, P. M., & Poretz, R. D. (1980) *Abstr. Pap. Int. Cong. Immunol. 4*, 19.5.8.

Laemmli, U. K., & Favre, M. (1973) *J. Mol. Biol. 80*, 575.

Miller, B. J., Hsu, R., Heinrikson, R., & Yachnin, S. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1388.

Murphy, L. A., & Goldstein, I. J. (1979) *Biochemistry 18*, 4999.

Noel, D., Nikaido, K., & Ames, G. F.-L. (1979) *Biochemistry 18*, 4159.

Rice, R. H., & Etzler, M. E. (1975) *Biochemistry 14*, 4093.

Waxdal, M. J. (1974) *Biochemistry 13*, 3671.

Yokoyama, K., Terao, T., & Osawa, T. (1978) *Biochim. Biophys. Acta 538*, *384*.