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Further characterization of monoclonal antibody 6,F-8 reacting with *Lathyrus, Lens* and *Pisum* lectins

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The murine monoclonal antibody (MoAB) 6,F-8 made against the glucose/mannose-specific *Lathyrus odoratus* mitogen has previously been shown to react with *Lens culinaris* and *Pisum sativum* lectins, but not with the lectin from *Vicia faba* [(1988) Biol. Chem. Hoppe-Seyler 369, 365–370]. The reactivity against seven other completely sequenced *Lathyrus* lectins has now been tested after separation of the subunits by SDS-polyacrylamide gel electrophoresis and electroblotting to nitrocellulose filters. Two of these lectins reacted with the antibody. Comparison of the amino acid sequences of the examined lectins and the predicted hydrophilic, flexible and accessible regions of *Pisum sativum* suggest that valine-147 is involved in antibody binding.

Lectin; Monoclonal antibody; Immunoblotting; (*Lathyrus, Lens, Pisum*)

1. INTRODUCTION

Most plant lectins are found in species belonging to the *Leguminosae* family. Plants from the *Vicieae* tribe, which include the genera *Lathyrus, Lens, Pisum* and *Vicia*, usually contain glucose/mannose-specific lectins composed of two light (M_r about 5000) and two heavy subunits (M_r about 18000). Comparison of the amino acid sequences of chains from different lectins has shown a high degree of homology (70–87%) [1]. Lectins from other tribes in this family are composed of a single type of subunit (M_r about 27000) associated to form tetramers with different sugar specificities. One-chain lectins as those from *Canavalia ensiformis* (Con A), and *Glycine max* (soybean) show a high degree of sequence homology (34–49%) [1] to the *Viciea* tribe lectins. The similarities in amino acid sequences among seed lectins from diverse leguminous species are predicted to reflect a conservation of basic secondary and tertiary structures [2]. Evolutionary substitutions may influence the antigenicity and the use of monoclonal antibodies (MoAb) has allowed a delineation of antigenic structures at a level of precision not possible with polyclonal antibodies.

Three murine IgG1 monoclonal antibodies have previously been made against the glucose/mannose-specific two-chain mitogen from seeds of *Lathyrus odoratus* (sweet pea) [3]. Two of these antibodies reacted with epitopes which by immunoblotting were shown to be expressed on all the examined one- and two-chain lectins. The third MoAb (6,F-8) reacted only with the heavy subunits from some two-chain lectins. Here we report immunoblot analyses with MoAb 6,F-8 for seven completely sequenced *Lathyrus* lectins.

2. MATERIALS AND METHODS

2.1. *Seeds and lectins*

Lathyrus aphaca, L. articulatus, L. cicera, L. nissolia, L. ochrus and *L. sphaericus* seeds were harvested from plants

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cultivated under field conditions in Toulouse, France. *Lens culinaris* (lentil) seeds were obtained from H.C. Schobbers BV, The Netherlands. Affinity chromatography on Sephadex G-100 columns were used for isolation of the *Lathyrus* and the lentil lectins. *Pisum sativum* and *Vicia faba* lectins were from E-Y, Lab. Inc., San Mateo, CA. *Vicia cracca* glucose/mannose-specific lectin was a gift from Dr Christian Baumann, Würzburg, FRG.

2.2. *Monoclonal antibody*

The preparation of MoAb 6,F-8 and the isolation of the IgG1 antibody from ascitic fluids have been reported [3].

2.3. *SDS-polyacrylamide gel electrophoresis and immunoblotting*

The electrophoresis was performed in a Laemmli system [4] with 7 × 8 cm and 0.75 mm thick slab gels containing 4 and 18% acrylamide in the stacking and separating gels, respectively. The lectins (4 μg) were not boiled and 2-mercaptoethanol was omitted. Immunoblotting was performed with Schleicher & Schuell nitrocellulose (NC) papers (0.2 μm) as described in [5]. The MoAb was used at a concentration of 1 μg/ml and peroxidase-conjugated rabbit immunoglobulins to mouse immunoglobulins (Dakopatts) at a dilution of 1:1000 in the presence of 0.1 M methyl-α-D-mannoside. The Pharmacia LMW calibration kit proteins were used as M_r standards. Control experiments were performed by Amido black staining of the NC papers with electroblotted proteins.

3. RESULTS AND DISCUSSION

MoAb 6,F-8 made against the glucose/mannose-specific two-chain lectin from *Lathyrus odoratus* (sweet pea) seeds [6,7] has previously been shown to react with an epitope expressed on the heavy chains from some *Vicieae* tribe lectins [3]. The antigenic determinant seemed to be sequential and not conformational since heating and SDS-treatment did not reduce the staining intensity. The reacting chains were from *L. odoratus, Pisum sativum* and *Lens culinaris* whereas *L. sativus* and *Vicia faba* were not stained on immunoblots. The reaction of the MoAb with seven other *Lathyrus* lectins were analyzed by immunoblotting (fig.1). The two strong-staining species were *L. aphaca* and *L. nissolia* (isolectin I). The five other *Lathyrus* lectins (*L. ochrus* (isolectin I), *L. ochrus* (isolectin II), *L. articulatus, L. cicera* and *L. sphaericus*) did not stain or only showed a very weak reaction with the antibody. A weak cross-reaction was seen at a higher MoAb concentration (10 μg/ml) for *L. ochrus* (isolectin I). All the ten

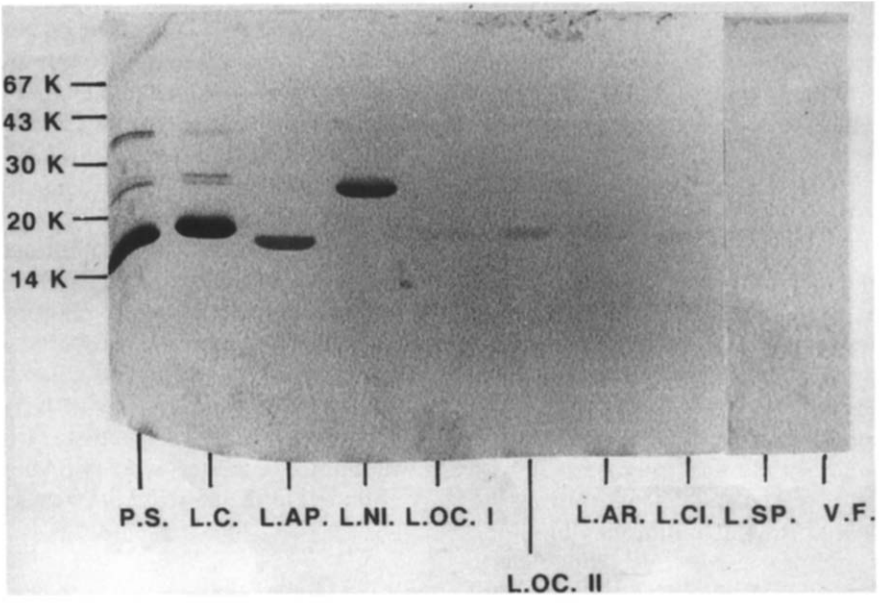

Fig.1. Immunoblot showing activity of MoAb 6,F-8 against lectins separated by SDS-PAGE and electroblotted to NC paper. The MoAb binding was detected with peroxidase-conjugated rabbit immunoglobulins to mouse immunoglobulins. The M_r markers were stained with Amido black. P.S., *Pisum sativum* lectin; L.C., *Lens culinaris*, lentil; L.AP., *Lathyrus aphaca*; L.NI., *Lathyrus nissolia*, isolectin I; L.OC. I and L.OC. II, *Lathyrus cicera*, isolectins I and II; L.AR., *Lathyrus articulatus*; L.CI., *Lathyrus cicera*, isolectin I; L.SP., *Lathyrus sphaericus*; V.F., *Vicia faba*, fava bean.

lectins (fig.1) are composed of heavy and light chains as generally found in the *Vicieae* tribe, except the antibody-reactive lectin *L. nissolia*, which contains single polypeptide chains of 233 or 235 amino acids [10] and the non-reactive chain from *L. sphaericus* which contains 244 amino acids [13]. The two atypical *Lathyrus* lectins show strong overlapping homology with both the heavy and light chains of the lectins in the *Vicieae* tribe.

The complete amino acid sequences are known for the ten examined chains and are shown in fig.2. A comparison between the strongly reacting and the weakly or non-reacting chains indicated that serine-12 or valine-147 are involved in the binding of MoAb 6,F-8. We also examined the binding to the glucose/mannose-specific lectin from *Vicia cracca* since the sequence of the 1–25 region showed that this heavy chain contained serine in position 12 [15]. The finding that this subunit did not stain by immunoblotting (results not shown) indicates that serine-12 is not involved in antibody binding.

Comparison with the sequences of glucose/mannose-specific lectins outside the *Vicieae* tribe

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                  1          10*                  20          30          40          50
 1. P.S.          TETTSFSLT KFSPD                QQNLI FQGDGYTTKE KLTLTKAVKN TVGRALYSSP IHIW
 2. L.C.          -------I- -----                ----- -------G-- G-----VS-E -G------T- ----
 3. L.AP.         -------I- -----                ----- --------S- ---------- ---------- ----
 4. L.NI.         ------LI- ---A-                ----- ---------D --L-----R- ---------- ----
 5. L.OC.I        -------I- --G--                ----- ---------- R-------R- ---------- ----
 6. L.OC.II       -------I- --G--                -P--- ---------- R-------R- ---------- ----
 7. L.AR.         -------I- --G--                ----- ---------- R-L-----R- ---------- ----
 8. L.CI.         ------L-- --G--                ----- ---------- R-------R- ---------- ----
 9. L.SP.      TE-------IP -TDQPSSPKFVSG-P--- ---NA-S-DG --I--E-KQ- --------A- ----
10. V.F.        TD-I----IP --R--               -P--- ---G------ ---------- --------L- ----

          60          70          80          90          100         110
 1. DRETGN VANFVTSFT F VINAPNSY  NV ADGFTFFIAP VDTKPQTGGG YLGVF NSAEY DKTTQTVAVE
 2. --D-V- ------NGSQV FRES--G-  -- ---------- ---------- -----Y-GK-- ---S------
 3. -SK--- ----I----- - -------- -- ---------- ---------- ----- --KD- ---SK-----
 4. -SQ--- --------- - -------- -- ---------- ---------- ----- --KD- ---SK-----
 5. -SK--- --------- - --D----- -- ---------- ---------- ----- --KD- ---S------
 6. -SK--- -------A- - --D----- -- ---------- ---------- ----- --KD- ---S------
 7. -SK--- --------- - --D----- -- ---------- ---------- ----- --KD- ---S------
 8. -SK--- --------- - -------- -- ---------- ---------- ----- --V-- ---S------
 9. --K--K --D-TA--- - Y-R ---DSQV- ---------- ---Q-RGD-- L---- -RE-- -P-IH-----
10. -S---- --D-T-T-I - --D---G-  -- ---------- ---------- ----- -GKD- ---A------

    120         130         140  *      150         160         170         180
 1. FDTFYNAAWD PSNRDRHIGI DVNSIKSVNT KSWKLQNGEE ANVVIAFNAA TNVLTVSLTY PNSLEEEN
 2. ---------- ---KE----- ---------- ---N----
 3. ---------- ---K------ ---------- --------K- ---------E ---------- S-
 4. ---------- ---G------ ---------- --------K- ---------- ---------- ---
 5. ------T--- ---G------ -------I-- --------K- ---------- ---------- --
 6. ------T--- ---G------ -------I-- --------K- --------G- ---------- --
 7. ------T--- ---G------ -------I-- --------K- ---------- ---------- --
 8. ---------- ----E----- -------I-- --------V- ---------- ---------- --
 9. ----H-QP-- -DY  I---V -I-----RI- RP-NPHYDTY SIAY--YK-- --E-D-TV-- --
10. ---------- ---GK----- ---T---IS- ---N------ -H-A-S---T ----S-T-L- --
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Fig.2. Amino acid sequences of the heavy subunits from *Pisum sativum* lecctin [8], *Lens culinaris* [9], *Lathyrus* lectins [10–13] and *Vicia faba* [14]. *Lathyrus nissolia* (isolectin I) and *Lathyrus sphaericus* are composed of single polypeptide chains and aligned from the N-terminal residues. The asterisks indicate the two residues that are identical in the four chains (P.S., L.C., L.AP. and L.NI) that bind MoAb 6,F-8, but different from those in the corresponding positions in the six non-reacting chains (L.OC. I, L.OC. II, L.AR., L.CI., L.SP. and V.F.). Lectin abbreviations are given in the legend to fig.1.

showed that the one-chain lectin from *Canavalia ensiformis* (Con A) has a serine residue in position 134 homologous to serine-12 of *Pisum sativum*, but a lysine residue at position 35 homologous to valine-147 of *Pisum sativum* [8]. Since Con A did not react with MoAb 6,F-8 [3], this too might indicate that the serine-12 residue was not involved in antibody binding.

The predicted hydrophilic, flexible and accessible regions of *P. sativum*, calculated by the methods described in [16–18], showed that valine-147 was well exposed, while serine-12 was not exposed. This also suggested that valine-147 was involved in the binding of MoAb 6,F-8.

The monoclonal antibody made against *Lathyrus odoratus* lectin recognized an epitope expressed in five of nine *Lathyrus* lectins, *Lens culinaris* and *Pisum sativum*. The epitope has not been detected in the genus *Vicia* of which *Vicia cracca* (glucose/mannose-specific) and *Vicia faba* were examined. Only the latter lectin has been completely sequenced within this genus. Our studies clearly showed that the epitope for MoAb 6,F-8 did not follow the borders for classical taxonomy within the *Vicieae* tribe. Further studies with this MoAb and other legume lectins are needed for conclusions about possible taxonomic relationships. The MoAb 6,F-8 is a useful antibody since it showed the same dot-blot staining intensity both with native and denaturated proteins [3].

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REFERENCES

[1] Yarwood, A., Richardson, M., Sousa-Cavada, B. and Rougé, P. (1985) FEBS Lett. 184, 104–109.
[2] Olsen, K.W. (1983) Biochim. Biophys. Acta 743, 212–218.
[3] Kolberg, J., Michaelsen, T.E., Wedege, E. and Heier, H.E. (1988) Biol. Chem. Hoppe-Seyler 369, 365–370.
[4] Laemmli, U.K. (1970) Nature 227, 680–685.
[5] Wedege, E. and Frøholm, L.O. (1986) Infect. Immun. 51, 571–578.
[6] Kolberg, J. and Michaelsen, T.E. (1979) Acta Pathol. Microbiol. Scand. Sect. C 87, 275–279.
[7] Kolberg, J., Michaelsen, T.E. and Sletten, K. (1980) FEBS Lett. 117, 281–283.
[8] Higgins, T.J.V., Chandler, P.M., Zurawski, G., Button, S.C. and Spencer, D. (1983) J. Biol. Chem. 258, 9544–9549.
[9] Foriers, A., Lebrun, E., Van Rapenbusch, R., De Neve, R. and Strosberg, A.D. (1981) J. Biol. Chem. 256, 5550–5560.
[10] Yarwood, A., Richardson, M., Morphet, B., Westby, M., Père, D. and Rougé, P. (1988) Phytochemistry 27, 1719–1721.
[11] Rougé, P., Richardson, M., Chatelain, C., Yarwood, A., Sausa-Cavada, B. and Père, D. (1986) in: Lectins Biology, Biochemistry, Clinical Biochemistry (Bøg-Hansen, T.C. and Van Driessche, E. eds) vol.5, pp.185–193, W. de Gruyter, Berlin.
[12] Yarwood, A., Richardson, M., Sousa-Cavada, B., Père D. and Rougé, P. (1986) Phytochemistry 25, 2109–2112.
[13] Richardson, M., Yarwood, A. and Rougé, P. (1987) FEBS Lett. 216, 145–150.
[14] Hopp, T.P., Hemperly, J.J. and Cunningham, B.A. (1982) J. Biol. Chem. 257, 4473–4483.
[15] Baumann, C., Rüdiger, H. and Strosberg, A.D. (1979) FEBS Lett. 102, 216–218.
[16] Parker, J.M.R., Guo, D. and Hodges, R.S. (1986) Biochemistry 25, 5425–5432.
[17] Karplus, P.A. and Schulz, P.E. (1985) Naturwissenschaften 72, 212–213.
[18] Emini, E.A., Hughes, J.V., Perlow, D.S. and Boger, J. (1985) J. Virol. 55, 836–839.