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Short communication

Insertion of pea lectin into a phospholipid monolayer

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Abstract

Pea lectin (PSL) is a secretory sugar-binding protein, readily soluble in aqueous solutions of low osmolarity. However, PSL also appears to be associated with the plasma membrane at the tip of young pea root hairs. By using the Wilhelmy plate method, we found that PSL can insert into a lipid monolayer. This property appeared to be independent of the sugar-binding ability of the protein. This result suggests that PSL may be directly involved in membrane-mediated interactions with saccharide ligands, for example during root hair infection by symbiotic rhizobia.

Pea lectin (PSL) is a non-enzymic sugar-binding protein with a molecular mass of 49 kDa. The three dimensional structure of PSL has been established [12, 13, 19], and appears to be very similar to that of other legume lectins studied. PSL is a dimeric protein, primarily made up of β-sheets and connecting loops (a so-called β-barrel protein). At the opposing ends of the dimer, each monomer contains one sugar-binding site with a preferential affinity for mannosides. PSL is abundantly present in pea seeds in which it is targeted to protein storage vacuoles [20]. Much less lectin is produced in other parts of the pea plants. From roots, PSL is secreted into the rhizosphere [6, 7, 9], where it contributes to accumulation of symbiotic *Rhizobium* bacteria on the tip of developing root hairs [17]. This lectin-mediated agglutination of bacteria is enabled by the dimeric structure of PSL and, consequently, its divalency of sugar binding.

In current hypotheses concerning the role of legume lectin in the plant or in the *Rhizobium*-legume symbiosis, the lectin invariably appears as a soluble protein, binding to cell surfaces via its sugar-binding site if appropriate. However, in pea roots PSL is also present on the outer surface of the plasma membrane in the growth area of root hairs and root hair-forming cells [6, 10, 11]. This membrane association is not abolished in the presence of haptenic sugars. Moreover, membrane-associated PSL is still able to bind a specific carbohydrate ligand [10, 11]. These observations suggest that membrane-association of PSL is possible without involvement of its sugar-binding sites. We tested this hypothesis by measuring PSL-lipid interactions by means of the monolayer technique [4]. This technique allows study of both binding and insertion of a protein in a lipid monolayer. We selected phosphatidylcholine (PC) as the standard lipid, since about

50% of the lipid fraction of plant membranes consists of PC [15].

PSL2, the final processed form of pea lectin in pea seeds and roots [16] was isolated from dry seed meal, and purified using affinity chromatography on Sephadex G-75 (Pharmacia, Uppsala, Sweden) and chromatofocussing, as described by Diaz *et al.* [9]. Recombinant PSL (wt PSL) and the non-sugar-binding PSL mutant N125D were isolated from *Escherichia coli* according to van Eijsden *et al.* [21]. L-α-phosphatidylcholine was obtained from Sigma (St. Louis, MO).

Monolayer surface pressure was measured by the (platinum) Wilhelmy plate method [3, 4] in a thermostatically controlled box, using a Cahn D202 microbalance. This method is based on determination of the weight of a small platinum plate which is partially submersed in an aqueous subphase. Differences in plate weight are directly related to surface pressure. The subphase was continuously stirred with a magnetic bar. If appropriate, PSL was added to the subphase through a hole in the edge of the dish. The pressure changes were followed until the surface pressure increase had reached a maximal value, usually within 30 min. Unless stated otherwise, the measurements were performed with the following parameters. The lipid monolayers were spread from a chloroform solution to give an initial surface pressure of about 22 mN/m on a sub-phase solution of 10 mM Tris hydrochloride, pH 7.5, used at 21.5 °C. As a standard, 80 μl from a 1 mg/ml PSL2 solution in the appropriate subphase (400 nM) was injected into the dish. The glass dish had a volume of 4 ml and a surface of 4.41 cm^2. Surface pressure increases of lipid monolayers after injection of proteins into the subphase can be interpreted as the result of actual insertion of the proteins between the phospholipids of the monolayer. Demel *et al.* [3] established that molecules known to interact with only the phospholipid head group did not induce such an increase in surface pressure.

Insertion of PSL2 into a PC monolayer was measured as a function of the amount of PSL2 injected into the sub-phase (Fig. 1). It could be established that PSL2 is surface-active and inserts into the PC monolayer. In the absence of a lipid monolayer, the surface pressure increase was 23 mN/m at most (data not shown). Surface activity was apparent at the same order of magnitude as, for instance, insertion of the membrane-associated protein SecA of *E. coli* [2]. Maximal activity was observed when at least 200 nM PSL2 was added to the subphase. In further experiments, 400 nM PSL2 were used since this concentration quickly yielded the maximal effect, in 15 to 30 min.

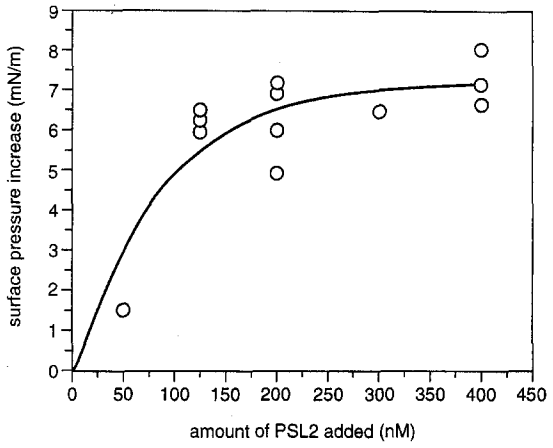

Fig. 1. Increase in the surface pressure of a monolayer of phosphatidylcholine as a function of the amount of pea lectin added.

Insertion of PSL2 could be detected up to an initial surface pressure of 35 mN/m (Fig. 2), which is beyond the assumed surface pressure of biological membranes [5]. The tetrameric soybean seed lectin, which has a monomer structure similar to that of PSL, showed a slightly smaller surface activity when tested under standard conditions at a concentration of 200 nM (Fig. 4). This may be due to differences in solubility and/or to the difference in multimerization.

Insertion could be enhanced by using the negatively charged lipid 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol (DOPG) instead of the zwitterionic PC, whereas monolayers of monoglucosyldiacylglycerol (MG1DG) or diglucosyldiacylglycerol (DG1DG) yielded slightly lower rates of insertion (data not shown). The latter observation suggests that possible binding of

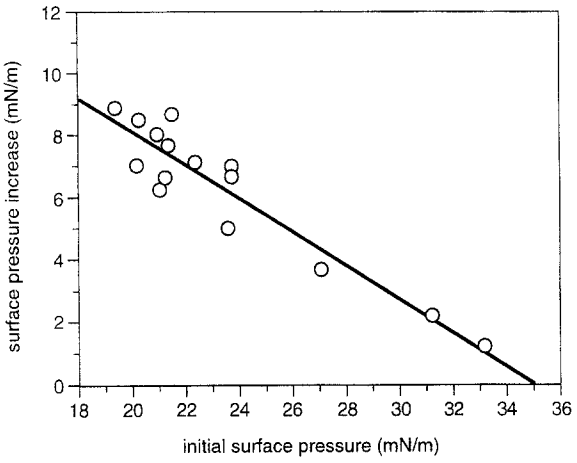

Fig. 2. Insertion of pea lectin into a phosphatidylcholine monolayer as a function of the initial surface pressure.

PSL2 to glucose residues of a glycolipid may inhibit rather than stimulate membrane insertion.

The effect of the pH on the initial insertion rate of PSL2 in a PC monolayer is shown in Fig. 3. Two different buffers were used to cover the pH range from values 3 to 9, a 10 mM sodium acetate buffer (pH 3 to 6) and a 10 mM Tris hydrochloride buffer (pH 6 to 9). The results show that the pH of the subphase significantly affects the initial insertion rate of PSL2. Insertion appeared to be slow at pH 6, which value is close to the pI of PSL2 (pH 6.1). This indicates that ionic interactions between PSL and the zwitterionic head group of PC may enhance membrane association of the protein. Eventually, the pH did not have a significant effect on total insertion of PSL2, although insertion tended to be larger at lower pH values (data not shown). Furthermore, high salt concentrations did not inhibit PSL insertion into the PC monolayer. These results suggest that insertion is primarily based on hydrophobic interactions, and that ionic interactions can speed up this process.

In order to test the influence of sugar-binding activity of PSL2 on membrane interaction, we tested (1) the influence of addition of 0.1 M mannose (a PSL ligand) or 0.1 M galactose (not a PSL ligand), and (2) surface activity of a PSL mutant incapable of binding monosaccharides [21]. Presence of mannose or galactose did not have any effect on PSL monolayer insertion. Furthermore, the non-sugar-binding mutant PSL N125D appeared to show the same membrane activity as did wild type PSL, both isolated from *E. coli* (Fig. 4). Both PSL preparations were slightly less active than was PSL2 isolated from pea seeds, which may be due to differences in solubility and/or to the fact that PSL from *E. coli* is not processed. These results strongly suggest that the PSL-lipid association essentially is inde-

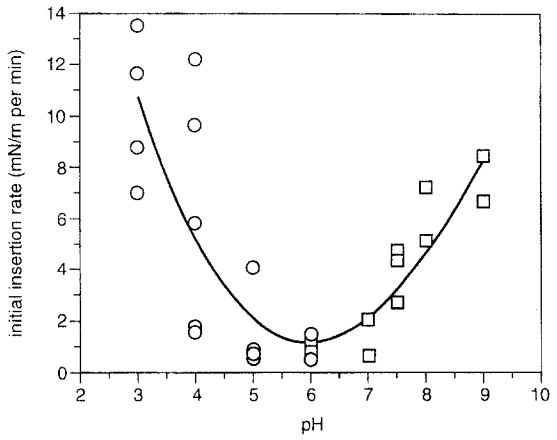

Fig. 3. Effect of the pH on the initial rate of insertion of pea lectin into a phosphatidylcholine monolayer. (○) Sodium acetate buffer, (□) Tris.Hcl buffer.

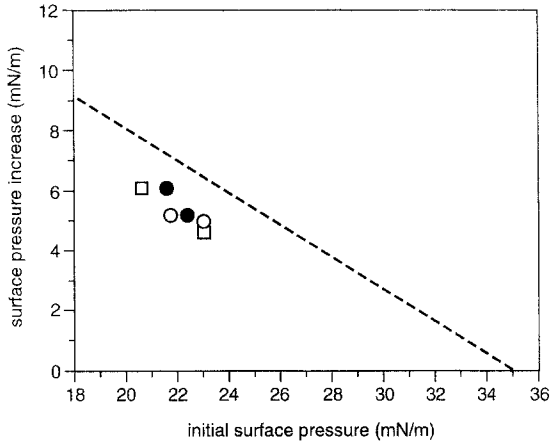

Fig. 4. Insertion of soybean seed lectin (●), a non-sugar-binding pea lectin mutant (□) and wild-type pea lectin (○) into a phosphatidylcholine monolayer. The broken line is derived from Fig. 2, to enable comparison with the situation for pea seed lectin.

pendent of the sugar-binding ability of the protein. However, use of a glycolipid rather than PC may modulate the results (as indicated above), dependent on the nature of the sugar moiety.

Our results may explain the presence of PSL2 at the plasma membrane of pea root hairs. Furthermore, the independence of surface activity from sugar binding is consistent with the observation that membrane-associated PSL is still able to bind a specific carbohydrate ligand [10, 11]. The PSL domains involved in membrane association remain to be determined. Like other legume lectins, PSL contains a conserved hydrophobic pocket [13] which may interact with lipids. Also the β-barrel structure of PSL may be an important factor for membrane association, as has been suggested for bacterial porins [18]. It is tempting to relate our results to the recent finding that legume lectins share homology with the membrane proteins VIP36 and ERGIC-53 [14], both presumable involved in the secretory pathway of animal cells. The results open the possibility that PSL is directly involved in the membrane-mediated interactions with specific saccharide ligands, for example during root hair infection by symbiotic rhizobia. Multivalency of such ligands may allow cross-linking of lectin-ligand complexes (for an example, [1]), leading to membrane changes and a modulation of cellular responses.

References

1. Bourne Y, Bolgiano B, Liao D-I, Strecker G, Cantau P, Herzberg O, Feizi T, Cambillau: Crosslinking of mammalian lectin (galectin-1) by complex biantennary saccharides. Nature Struct Biol 1: 863–870 (1994).
2. Breukink E, Demel RA, de Korte-Kool G, de Kruijff B: SecA insertion into phospholipids is stimulated by negatively charged lipids and inhibited by ATP: a monolayer study. Biochemistry 31: 1119–1124 (1992).
3. Demel RA: Monolayers. Description of use and interaction. Meth Enzymol 32: 539–545 (1974).
4. Demel RA: Monomolecular layers in the study of biomembranes. Subcell Biochem 23: 83–120 (1994).
5. Demel RA, Geurts van Kessel WSM, Zwaal RFA, Roelofsen B, van Deenen LLM: Relation between various phospholipase actions on human red cell membranes and the interfacial phospholipid pressure in monolayers. Biochim Biophys Acta 406: 97–107 (1975).
6. Diaz CL, van Spronsen PC, Bakhuizen R, Logman GJJ, Lugtenberg EJJ, Kijne JW: Correlation between infection by *Rhizobium leguminosarum* and lectin on the surface of *Pisum sativum* L. roots. Planta 168: 350–359 (1986).
7. Diaz CL: Root lectin as a determinant of host-plant specificity in the Rhizobium-legume symbiosis. Ph.D. thesis, Leiden University, Netherlands (1989).
8. Diaz CL, Melchers LS, Hooykaas PJJ, Lugtenberg BJJ, Kijne JW: Root lectin as a determinant of host plant-specificity in the *Rhizobium*-legume symbiosis. Nature 338: 579–581 (1989).
9. Diaz CL, Hosselet M, Logman GJJ, van Driessche E, Lugtenberg BJJ, Kijne JW: Distribution of glucose/mannose specific isolectins in pea (*Pisum sativum* L.) seedlings. Planta 181: 451–461 (1990).
10. Diaz CL, Hooykaas PJJ, Lugtenberg BJJ, Kijne JW: Functional expression of pea lectin on root hair tips of transgenic white clover hairy roots. In: Gresshoff PM, Roth LE, Stacey G, Newton WE (eds) Nitrogen Fixation: Achievements and Objectives, p. 733. Chapman and Hall, New York/London (1990).
11. Diaz CL, Logman GJJ, Stam JC, Kijne JW: Sugar-binding activity of pea lectin expressed in white clover hairy roots. Plant Physiol 109: 1167–1177 (1995).
12. Einspahr H, Parks EH, Suguna K, Subramanian E, Suddath FL: The crystal structure of pea lectin at 3.0 Å resolution. J Biol Chem 261: 16518–16527 (1986).
13. Einspahr H, Parks EH, Phillips SR, Suddath FL: Crystal structure studies of legume lectins. In: Bog-Hansen TC, Freed DLJ (eds) Lectins: Biology, Biochemistry, Clinical Biochemistry, vol. 6, pp. 245–263. Sigma Chemical Company, St. Louis, MN (1988).
14. Fiedler K, Simons K: A putative novel class of animal lectins in the secretory pathway homologous to leguminous lectins. Cell 77: 625–626.
15. Harwood JL: Plant acyl lipids: structure, distribution and analysis. In: Stumpf PK (ed) The biochemistry of plants, vol. 4. Lipids: Structure and Function. pp. 1–55. Academic Press, New York (1980).
16. Hoedemaeker PhJ, Richardson M, Diaz CL, de Pater BS, Kijne JW: Pea (*Pisum sativum* L.) seed isolectins 1 and 2 and pea root lectin result from carboxypeptidase-like processing of a single gene product. Plant Mol Biol 24: 75–81 (1994).
17. Kijne JW, Smit G, Diaz CL, Lugtenberg BJJ: Lectin-enhanced accumulation of manganese-limited *Rhizobium leguminosarum* cells on pea root hair tips. J Bact 170: 2994–3000 (1988).
18. Lugtenberg BJJ: Structure and function of outer membrane proteins. In: Korhonen TK, Dawes EA, Makela PH (eds) Enterobacterial Surface Antigens: Methods for Molecular Characterization, pp. 1–16. Elsevier Science Publishers, Amsterdam (1985).
19. Rini JM, Hardman KD, Einspahr HM, Suddath FL:

Solution of a pea lectin-trimannoside complex by molecular replacement. Trans Am Cryst Ass 25: 51–63 (1991).
20. van Driessche E, Smets G, Dejaegere R, Kanarek L: The immuno-histochemical localization of lectin in pea seeds (*Pisum sativum* L.). Planta 153: 287–296 (1981).
21. van Eijsden RR, Hoedemaeker FJ, Diaz CL, Lugtenberg BJJ, de Pater BS, Kijne JW: Mutational analysis of pea lectin. Substitution of Asn^{125} for Asp in the monosaccharide-binding site eliminates mannose/glucose-binding activity. Plant Mol Biol 20: 1049–1058 (1992).