J. Cell Sci. **42**, 417–423 (1980)
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POLLEN-STIGMA INTERACTION IN *BRASSICA OLERACEA*: THE ROLE OF STIGMATIC PROTEINS IN POLLEN GRAIN ADHESION

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SUMMARY

The adhesion of pollen grains to the stigmas of *Brassica oleracea* was assayed after treatment of the stigmas with protease and/or cycloheximide. Treatment with protease alone adversely affected pollen grain adhesion. However, the adhesive properties of the stigma recovered fully if the stigmas were not pollinated until 2 h after treatment. Immersion of the stigmas in cycloheximide after protease treatment prevented any recovery of the stigmas' adhesive properties. Cycloheximide treatment alone prevented pollen grain adhesion when pollination occurred later than 1–2 h after treatment but did not affect pollen grain adhesion if pollination occurred immediately after treatment. These results indicated not only that the surface-held proteins of the stigma are involved in pollen grain adhesion, but also that their turnover rate is rapid. Isoelectric focusing of extracts derived from stigmas after protease and cycloheximide treatment showed a marked decrease in staining intensity of 3 protein bands, one of which, a glycoprotein, is known to be present only when the self-incompatibility system is fully functional. These observations suggest a specificity of adhesion between higher plant cells in the presence of the cell wall.

INTRODUCTION

Proteins, and particularly glycoproteins, have long been known to be involved in adhesion between cells. Most reports, however, have been concerned with animal cells, whilst studies of adhesion between higher plant cells are infrequent and usually deal either with isolated protoplasts or membrane fractions (Larkin, 1978). The possibility of plant cells adhering to one another in the presence of the cellulosic wall seems not to have been investigated previously. Despite this lack of reports concerning cell-cell binding in plants there is much evidence that proteins do exist within the plant cell wall, and some of these have been shown to possess carbohydrate-binding capabilities (Kauss & Bowles, 1976).

Surface proteins have already been shown to be important in the reproductive processes of lower plants, especially in the unicellular green alga, *Chlamydomonas* (Weise, 1974; Snell, 1976) and more recently the multicellular brown alga, *Fucus serratus* (Bolwell, Callow, Callow & Evans, 1979). Reports of the role of surface pro-

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teins from higher plants are far fewer, but in the Caryophyllaceae, enzymic removal of the stigmatic pellicle by protease prevents pollen tube penetration without affecting pollen grain germination (Heslop-Harrison & Heslop-Harrison, 1975). It has also been shown that the stigma surfaces of a number of species are able to bind the lectin, Concanavalin A (Con A) and, in at least one species, *Gladiolus*, pollen tube penetration is prevented if the receptor sites for Con A are occupied (Knox *et al.* 1976).

It has been suggested that the proteinaceous pellicle overlying the stigmatic papillae may contain the molecules responsible for recognition of pollen in species with a sporophytic self-incompatibility (SI) system (Mattsson, Knox, Heslop-Harrison & Heslop-Harrison, 1974). It would also seem likely that a component of this pellicle is responsible for the differences in the adhesion of pollen grains from compatible and incompatible sources that are observed immediately after pollination (Stead, Roberts & Dickinson, 1979). In this paper we report the effect of removing elements of the pellicle on the adhesion of compatible pollen and the possible role of the stigmatic glycoprotein that appears concomitant with the development of the SI system (Roberts, Stead, Ockendon & Dickinson, 1979).

MATERIALS AND METHODS

Flowering plants of *Brassica oleracea*, of known S-genotype, were kindly supplied by Dr D. J. Ockendon from N.V.R.S., Wellesbourne, Warwick, U.K. These plants were maintained in a growth room at 10 °C with a day length of 16 h.

Pollen grain adhesion

Flower buds (homozygous for the A16 S-allele) that were about to open (i.e. just prior to anther dehiscence) were excised from the plant and dissected in such a way that the stigma could be immersed in the experimental solutions. The stigmas were cross-pollinated with pollen from a plant homozygous for the S-allele S39 at various time intervals after treatment for 10 min with either protease (non-specific, from *Streptomyces griseus*) (1 mg ml^{-1} in 0·1 M Tris-HCl buffer, pH 7·4); protease followed by cycloheximide (2×10^{-4}M in the same buffer); or cycloheximide alone. Control stigmas were cross-pollinated without treatment or after immersion in buffer alone. All chemicals were obtained from Sigma (U.K.). The pollinated stigmas were left for 45 min after which time the adhesion of the pollen grains was assayed as described elsewhere (Stead *et al.* 1979).

Isoelectric focusing of stigma extracts

Stigmas were immersed in the solutions detailed above for various lengths of time (see text for details), washed in buffer and then extracted in a minimum volume of 0·1 M Tris-HCl buffer (pH 7·4) at 0 °C. Treatment and preparation were otherwise as described by Roberts *et al.* (1979).

RESULTS

After cross-pollinating untreated stigmas 29·3% of the pollen grains could be removed by the assay method; similarly 33·5% of the pollen grains could be removed from stigmas that had been treated with buffer alone. However, after immersing the stigma in a protease solution for 10 min the pollen grains adhered so poorly that approximately 80% of the pollen grains could easily be removed. In those stigmas

that had been treated with both cycloheximide and protease the percentage of pollen grains released remained high, even if the stigmas were left for 2·5 h after treatment prior to pollination (Fig. 1 A). When treated with protease alone the ability of the pollen grains to adhere to the stigma increased if an interval elapsed after immersion in protease. In fact, if left for between 1·0 and 1·5 h the adhesive properties of the stigma recovered fully (Fig. 1 C). Treatment with cycloheximide alone increased only slightly the percentage of pollen grains released relative to the controls, when pollination occurred immediately after the treatment. However, if the stigmas were left after treatment, the pollen grains failed to adhere. Indeed, if the stigmas were left for 2 h after treatment before pollinating, the pollen grains failed to adhere any better than in those stigmas treated with protease and cycloheximide (Fig. 1 B).

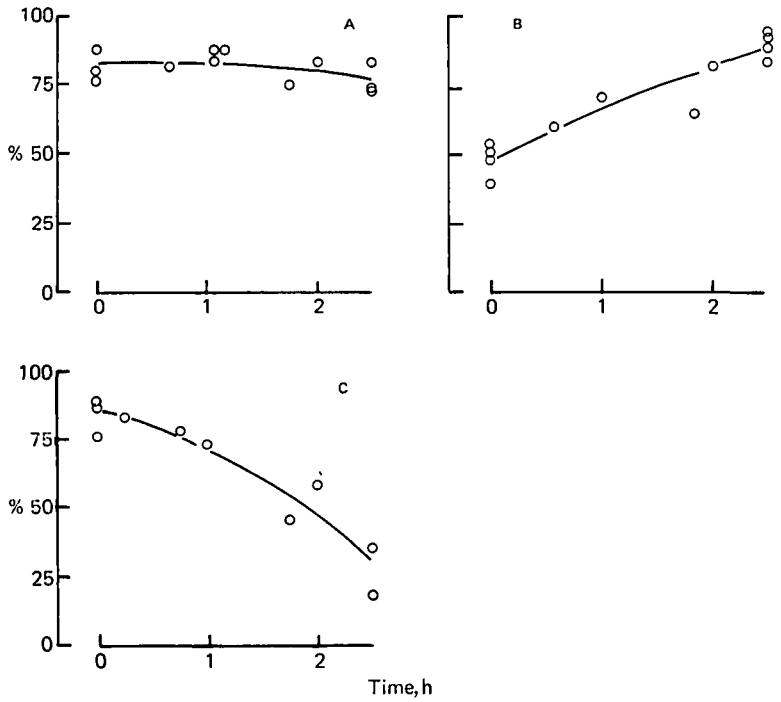

Fig. 1. The adhesion of compatible pollen grains, 45 min after pollination, expressed as a percentage of the final number of pollen grains released by 65 s of stirring, at various times after treating the stigma with: A, protease (10 min) followed by cycloheximide (10 min); B, cycloheximide (10 min); and C, protease (10 min).

Protease treatment alone for 10 min did not alter the composition of the stigma proteins after isoelectric focusing, nor did treatment with protease (10 min) followed by cycloheximide (10 min) significantly alter the protein profiles. However, when stigmas were left for 1 h after immersion in protease (10 min) and cycloheximide (10 min) and then treated again with protease (10 min) to remove any remaining surface protein the differences in the protein profiles were considerable (Fig. 2A, B). The intensity of at least 3 peaks was much reduced (labelled *a–c*), whilst that of a fourth was increased (labelled *d*).

DISCUSSION

Since immersion of the stigmas in a solution of protease, prior to pollination, strongly inhibits the subsequent adhesion of pollen grains, the proteins of the stigmatic surface must be involved in the adhesion of the pollen grains. Furthermore, if protein synthesis is inhibited by immersion in cycloheximide after protease treatment

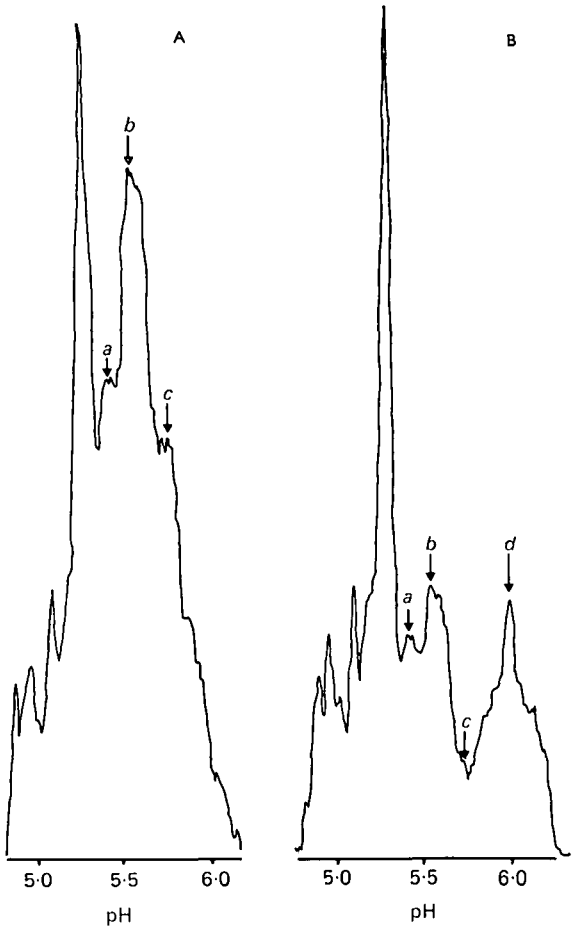

Fig. 2. 'Chromoscan' traces of isoelectric focusing gels loaded with extracts of stigmas just prior to flower opening and stained with Coomassie Brilliant Blue R250. A, extract of stigmas immersed in buffer only; B, extract of stigmas immersed in protease (10 min) cycloheximide (10 min) and finally in protease (10 min) again 1 h later. Three distinct bands (*a–c*) at least, are reduced by the treatment and a fourth (*d*) is increased. The pH gradient is indicated on the bottom scale.

the adhesive properties of the stigma do not recover, whereas after protease treatment alone, the stigmas fully recover their adhesive properties if they are left for between 1·5 and 2·5 h before pollination. Treatment with protease may of course digest more than just the superficial layers of the stigmatic papillae. This seems unlikely, however, as little or no difference could be detected in the ultrastructure of the papillar cells

after protease treatment (unpublished data). Furthermore, since the papillae are still capable of protein synthesis after protease digestion (as shown by the renewed ability to bind pollen grains) the cells are hardly likely to have been totally disrupted. In this connexion Heslop-Harrison & Heslop-Harrison (1975) also reported that stigmatic papillae of *Agrostemma* were not adversely affected by up to 2 h digestion with a crude protease enzyme.

Since 3 proteins appear to be affected by the protease and cycloheximide treatment it may be that these are the proteins responsible for pollen grain adhesion. In particular one of the protein bands (labelled *c*) corresponds to the glycoprotein shown by Roberts *et al.* (1979) to be present only when the SI system is fully functional. Furthermore in young buds the stigmas, from which this glycoprotein is absent, are incapable of binding pollen regardless of the compatibility (unpublished data). However, while it would be easy to assume that this protein, at least, is responsible for the adhesion of pollen grains, it should be emphasized that other features of the young stigmas may prevent pollen grain adhesion. For example, the papillae of young stigmas are densely packed and therefore pollen grains can make contact with only the very tips of the papillae, whereas in older flowers the papillae are much more open and pollen grains may lodge between individual papillae (Ockendon, 1972).

The low levels of the glycoprotein (labelled *c* in Fig. 2A) associated with the development of the SI system, relative to those shown previously (Roberts *et al.* 1979) almost certainly results from a necessity to use somewhat younger material for these experiments. In the buds the synthesis of this particular glycoprotein was probably incomplete. The failure to alter the stigma protein profile significantly by treatment with protease alone, or indeed with protease followed by cycloheximide, whilst similar treatments adversely affected pollen grain adhesion, is thought to result from large reserves of the relevant proteins being present within the papillae. These reserves of protein would not be immediately available to bind pollen grains but, since isoelectric focusing was performed on stigma homogenates, they would nevertheless be extracted from the tissue. However, by preventing protein synthesis, allowing the reserves to be secreted to the surface, and finally removing any surface protein with protease, the protein composition was significantly changed. This suggests that the stigma surface proteins have a rapid turnover rate.

Indeed, the data from the pollen grain adhesion studies also suggest that the turnover rate is rapid. Treatment with cycloheximide alone suggests that the proteins have a half-life of only a little over 1 h. After treatment with buffer alone approximately 30% of the pollen grains were easily removed, compared with a maximum percentage of approximately 90% after treatment with protease or protease plus cycloheximide. Therefore a value of 60% for the pollen grains released is intermediate and may correspond to a loss of about half of the effective stigma surface proteins. If this is so then pollination 30 min after treatment with cycloheximide alone produces an estimate of the proteins' half-life. However, since pollen grain adhesion was assayed 45 min after pollination, the half-life is in fact 75 min (assuming a continuation of protein turnover regardless of the presence of pollen grains). This relatively fast turnover rate for the protein is supported by the observation that it takes between 90 and 135 min

for the stigmas to recover half of their adhesive properties after protease treatment alone. Such fast turnover rates for plant proteins are rare, although common in animals; indeed the surface-held proteins of animals that are responsible for agglutination often have similar turnover rates. For example, following treatment with trypsin, the cell surface glycoproteins responsible for cell adhesion of chick embryo fibroblasts are restored within 1·5 h (Vernay, Cornic, Aubrey & Bourrillon, 1978).

Whilst the ability of animal cells, and more latterly plant protoplasts, to bind and agglutinate has been demonstrated, this is the first report of higher plant cells binding to one another with the cell wall still intact. It should, however, be appreciated that on the pollen grain at least the factors responsible for the binding are entirely located within the exine cavities. Furthermore, they are derived not from the pollen grain itself but from the tapetum and transferred to the outside of the pollen grain prior to anther dehiscence and must therefore be considered to be extracellular (Dickinson & Lewis, 1973). The proteins of the stigma that are responsible for pollen grain adhesion are similarly situated outside the protoplast. However it has been shown that, unlike most other higher plant cells, the stigmatic papillae produce a proteinaceous pellicle (Mattsson *et al.* 1974) over the cell wall, and it is presumably within this that the proteins responsible for pollen grain adhesion are situated.

We would like to thank the ARC and SRC for financial support and NVRS, Wellesbourne for the provision of plant material.

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(*Received* 30 *July* 1979)