Evidence of Penetration of $^{125}$I-labelled Tobacco Mosaic Virus Antibody into Fixed Isolated Plant Protoplasts

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Intact plant cells are impermeable to both ferritin conjugated antibodies (Shalla & Amici, 1967) and the considerably smaller iodine labelled antibodies (Langenberg & Schlegel, 1967). During a preliminary study of the use of $^{125}$I-labelled antibody in studies of tobacco mosaic virus infection evidence has been obtained for the penetration of intact fixed isolated tomato fruit protoplasts by iodinated antibodies.

$^{125}$I-labelled tobacco mosaic virus antibody was prepared by iodinating a tobacco-mosaic-virus-antibody precipitate with carrier-free sodium iodide [$^{125}$I] using chloramine, following methods described by Langenberg & Schlegel (1967) and McConahey & Dixon (1966). After releasing virus from the iodinated precipitate with 0.1 M glycine, pH 2.5, the antibody solution was dialysed against saline neutral phosphate buffer (0.9 % sodium chloride in 0.01 M phosphate buffer, pH 7.0, containing 0.01 M sodium azide) to remove residual iodide. Protoplasts were isolated from fully infected tomato locule tissue (Cocking & Pojnar, 1968), fixed in 6 % glutaraldehyde (0.05 M sodium phosphate buffer, pH 7.0) for 3 hr and washed three times with saline neutral phosphate to remove free fixative. The protoplasts were then incubated with the $^{125}$I-labelled antibody first for 2 hr at 18 ° and then for 16 hr at 4 ° in an attempt to facilitate penetration of the labelled antibody under conditions least likely to cause its degradation. After extensive washing with numerous changes of saline neutral phosphate buffer until no further free antibody was being removed (deduced by scintillation counting), the protoplasts were postfixed in 2 % osmium tetroxide (0.1 M sodium phosphate in 10 % sucrose, pH 7.0), double stained during dehydration with phosphotungstic acid (1 %, 10 min.) and uranyl acetate (1 %, 1 hr) in absolute ethanol (Mayo & Cocking, 1968) and embedded in n-butyl methacrylate-styrene (7:3 v/v) (Mohr & Cocking, 1968). Silver-gold sections were mounted on molybdenum grids and coated with a thin layer of carbon. The grids were then individually coated with monolayers of Ilford L4 emulsion gelled in a loop (Caro & van Tubergen, 1962). After storage for several weeks at 4 ° the grids were developed with D19B or Microdol X, and examined in an AEI EM6B electron microscope operating at 60 kv using a 50 μm. objective aperture.

Following incubation in labelled antibody, radioactivity was detected in the cytoplasm of protoplasts as in Pl. 1 a, most frequently in association with areas of recognizable virus particles (Pl. 1 b, c). It would appear likely that $^{125}$I-labelled antibody had penetrated the protoplasts and reacted with virus, resulting in the localization of radioactivity. However, there is the possibility that iodine unassociated with antibody may also be localized in these regions. Thus, while these present results provide some evidence for the penetration of iodinated antibody further studies using iodinated globulins from normal serum will be required to obtain more unequivocal evidence.

The removal of the plant cell wall during protoplast preparation appears to have eliminated a major penetration barrier. Fixed isolated protoplasts seem therefore to
be similar to certain fixed animal cells (Pierce, Ram & Midgeley, 1964) in that they are permeable to substantially unaltered antibody molecules whilst not to larger molecules such as ferritin (DeLeo & Cocking, 1967). Protoplasts therefore offer the possibility of investigating antigen distribution in infected cells using an intact system rather than freeze-damaging or sectioning specimens to allow antibody penetration (cf. Shalla & Amici, 1967). Several plant tissues (Ruesink & Thimann, 1966), including tobacco leaf (Takebe, Otsuki & Aoki, 1968), may be used to prepare isolated protoplasts, and it seems likely therefore that a wide variety of virus infections can be studied utilizing antibody penetration of fixed isolated protoplasts.

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REFERENCES


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