Incorporation of antibodies into tobacco cells treated with solutions similar to vitrification solutions

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Introduction

The plasmalemma and cell wall of plant cells control the transport and entry of large molecules. Transient and reversible permeabilization of this barrier, if possible, would offer several useful applications such as locating macromolecules and blocking receptors using specific antibodies. Permeabilization of plant cells has been attempted using chemical and physical treatments (Felix 1990, 1992). Some organic solvents successfully permeabilized cells but cell viability tended to be rather low (Felix 1990). Methods that could permeabilize cells only transiently without hindering cell viability and growth are ideal.

In the process of developing new cryoprotective solutions for vitrification methods, we found that these solutions made cells considerably leaky, which hinted that they could be used for cell permeabilization. We have recently found that solutions similar to vitrification solutions in composition can permeabilize suspension-cultured cells (Tandon *et al.* 1999). Here we report the essence of the work: a transient cell permeabilization procedure for high incorporation of fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin G (FITC–IgG) by the use of cocktails comprising glycerol, sucrose, ethylene glycol and DMSO and indicate the potential for a similar phenomenon happening during treatment with vitrification solutions made up of similar ingredients.

Materials and methods

The tobacco (*Nicotiana tabacum*) BY–2 cell line was harvested on the 4th day of weekly subculture and used as detailed elsewhere (Tandon *et al.* 1999).

About 10 mg cells were permeabillzed using 100 µl of cell permeabilization solutions (CPS) as detailed in Table 1, containing 1 µl of FITC–IgG (FITC anti-mouse IgG; 150 kDa) for 15 s at room temperature and then immediately washed twice with 1 ml of ice-cold 1M sucrose solution containing 10 mM $CaCl_2$ and then once with 1 ml of modified LS medium. The cells were pelleted by centrifugation at 3000 rpm for 5 min at 0°C in between the washings. As varying factors, different permeabilization solutions, duration of permeabilization, addition of $CaCl_2$ in CPS, cell age and different diluting conditions were tested for their effects on incorporation of FITC–IgG into tobacco cells and cell viability.

Cell viability was determined by staining with fluorescein diacetate (FDA). For both viability measurements and incorporation of FITC–IgG, about 5000 cells were observed for each treatment under UV-microscopy. The control cells showed only marginal autofluorescence during the 7-d culture period.

Results and discussion

Of the permeabilization solutions used, a fine balance of glycerol, sucrose, ethylene glycol and DMSO (20:5:20:5 w/v%) present in CPS–3 was most effective in incorporating high molecular weight FITC–IgG and at the same time retaining cell viability. From Table 1, it is apparent that the presence of both ethylene glycol and DMSO in the permeabilization solution was essential for better uptake and cell survival.

The optimal duration of permeabilization for both higher incorporation of FITC–IgG and survival of the cells was obtained using a 15-s incubation. The addition of 10 mM $CaCl_2$ to CPS–3 resulted in improved uptake of FITC–IgG and cell survival. The highest incorporation of FITC–IgG and cell survival were observed with cells harvested on the 4th day of culture.

Following the permeabilization and washing procedures described above, the morphology of the cells remained intact and there was no lysis of cells or destruction of subcellular organization. The permeabilized cells showed fluorescence from FITC–IgG in the peripheral and nuclear regions as well as in cytoplasmic strands. It was confirmed that fluorescence was located only in the cytoplasm and not in the wall and that plasma membrane impermeability to osmoticum (1M sucrose) was recovered showing plasmolysis similar to untreated control cells. The permeabilized cells retained high viability, as determined by FDA staining (data not shown).

Table 1. Incorporation of FITC–IgG into tobacco cells and cell survival using different cell permeabilization solutions: comprising glycerol (Gly), sucrose (Suc), ethylene glycol (EG), dimethyl sulfoxide (DMSO) and 40 mM $CaCl_2$. About 10 mg of cells were permeabilized using 100 µl of CPS containing I µl of FITC–IgG for 15 s at room temperature and then immediately washed twice with 1 ml of ice-cold 1M sucrose solution containing 10 mM $CaCl_2$ and then once with 1 ml of modified LS medium. The data are the mean ± SE (n=50).

Permeabilization solution	Concentrations (w/v%)				Cells showing FITC–IgG incorporation (%)	Cell survival assessed from FDA staining (%)
	Gly	Suc	EG	DMSO		
Control [1]	–	–	–	–	0	98.0 ± 4.1
CPS-1	20	5	30	10	93.9 ± 2.8	21.4 ± 1.7
CPS-2	20	5	25	5	87.4 ± 3.4	37.0 ±1.5
CPS-3	20	5	20	5	76.4 ± 2.6	78.2 ± 2.5
CPS-4	20	10	10	5	58.3 ± 1.9	56.8 ± 3.2
CPS-5	20	5	20	0	49.6 ± 1.6	76.0 ± 2.9
CPS-6	20	10	0	10	65.7 ± 1.8	68.1 ± 4.3
CPS-7	20	5	0	5	60.0 ± 2.0	77.3 ± 1.4

[1] Control cells were suspended in modified LS medium with FITC–IgG and washed with the medium.

Regrowth of the permeabilized cells was 75–80% of untreated control cells after 21 d of culture. A weak fluorescence from incorporated FITC–IgG was noticeable in the dividing cultured cells for about 7 d, which faded later (data not shown).

The permeabilization shown here may involve permeabilization of the cell wall and plasmalemma resulting in pore formation in the membrane, followed by the uptake of FITC–IgG through hypertonic and/or hypotonic shock. While glycerol and sucrose in CPS may provide needed osmotic stress, ethylene glycol and DMSO bring about permeation of the cell wall and plasma membranes. The organization of the pectic substances is a major controlling element in defining the sieving properties of the cell wall (Baron–Epel *et al.* 1988). DMSO is often used for solubilization of polysaccharides such as pectin from the cell wall and is also known to increase membrane fluidity. The presence of 10% glycerol has been reported to completely inhibit the release of cellular materials and enzymes (Felix 1992).

In conclusion, the procedure described herein is a reversible type of cell permeabilization that allows incorporation of antibodies and possibly even larger molecules. Various applications may be expected such as locating macromolecules and blocking receptors with specific antibodies. We expect similar cell permeabilization in cells treated with vitrification solutions such as PVS2 (Sakai *et al.* 1990) as the composition is similar to the solutions used in this study.

References

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