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Effect of Temperature on Membrane Fluidity and Protoplast Fusion

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Summary. The fusion of plant protoplasts is greatly enhanced by a rise in temperature in the presence of the fusion-inducing agents polyéthylene glycol or polyvinyl alcohol. Kinetic analysis of this reaction reveals that membrane fluidity is important in regulating the fusion process.

Key words: Flow activation energy – Membrane fluidity – Protoplast fusion – *Rauwolfia serpentina* var. 'Bentham' – Temperature

Introduction

Raising the temperature in fusion experiments has been reported to facilitate the fusion of animal cells (Toister and Loyter 1973; Ahkong et al. 1975; Okada 1976) and that of plant protoplasts (Keller and Melchers 1974), but there are only two studies on why this is so (Ahkong et al. 1973; Prives and Shinitzky 1977). Kinetic analysis of the rate of fusion is necessary to show that membrane fluidity is important for protoplast fusion (Prives and Shinitzky 1977; Yamada et al. 1979). We here report that the fusion of plant protoplasts is greatly enhanced by a rise in temperature in the presence of the fusion-inducing agents polyethylene glycol (PEG) or polyvinyl alcohol (PVA), and present kinetic analysis of this reaction.

Material and Methods

Cells of *Rauwolfia serpentina* var. 'Bentham' were cultured, and protoplasts were prepared as described in a previous paper (Yamada and Nakaminami 1973) with modifications (Cellulase Onozuka R10 1%, Macerozyme 0.2%, potassium dextran sulfate 0.5%, sorbitol 0.6 M). About two hours after preparation, protoplasts were used in fusion experiments in a jacketed cell that could be observed under a light microscope. Water was circulated through the jacket at controlled temperatures. Protoplasts were suspended in 0.6 M sorbitol containing 10 mM $CaCl_2$ (pH 5.6, not adjusted) at a density of about 10^5 protoplasts per milliliter. Two drops of this suspension were placed in the jacketed cell and mixed with eight drops of a solution containing the fusion-inducing chemicals, PEG ($\overline{DP}$ = ca. 220) or PVA ($\overline{DP}$ = ca. 500) in 0.6 M sorbitol and 10 mM $CaCl_2$. The mixture then was incubated for 60 minutes at $2 \pm 1°C$, after which the incubation temperature was raised to the desired degree by changing the temperature of the circulating water; the time lag was less than 10 minutes.

Results

The fusion process, observed under a microscope, showed that first, two or more protoplasts come into contact and adhere at a point, and that each adhering-protoplast remains spherical. This we call the 'point-adherence' stage. In the absence of PEG, but with calcium ion, protoplasts remain at this stage and do not proceed to the second stage, although the number of protoplasts at the point-adherence stage increases with time. In the presence of PEG the point adherence protoplasts pass to the 'face-adherence' stage, in which two protoplasts are joined by a large surface area so that the initially spherical protoplast cells become deformed. This stage is sometimes called the 'fused-membrane' stage (Okada 1976), and implies that membrane fusion indeed has been induced (Poste and Alison 1973; Poste 1974). The face-adherent protoplasts subsequently proceed to the 'fused-sphere' stage, in which two protoplasts have united to form a single protoplast of spherical or oval shape. The extent of adhesion or fusion of the protoplasts is expressed as the number of adherent or fused protoplasts per hundred of the total number of protoplasts. This was plotted against incubation time; three of these plots are shown in Figure 1.

As stated above, in the presence of a fusion-inducing agent point-adherence protoplasts passed to the face-adherence stage, the number of face-adherence protoplasts increasing with incubation time. This increase in the num-

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ber of face-adherence protoplasts was enhanced markedly by raising the incubation temperature (Fig. 1). The rate of increase in the number of fused-sphere protoplasts also was enhanced by raising incubation temperature, though less markedly than in the case of face-adherence protoplasts. That the number of face-adherence protoplasts increased initially, then subsequently decreased as the number of fused-sphere protoplasts became appreciable, indicates that the plots may be analyzed by the kinetic theory of a consecutive reaction (Moelwyn-Hughes 1961): point-adherence protoplast, face-adherent protoplast and fused-sphere protoplast, though with rigor the kinetics of formation of point-adherence protoplast should be taken into consideration. In this study, ease of fusion due to a rise in temperature was conveniently evaluated by measuring the 'time of fusion' (Ahkong et al. 1973), $\underline{t}^{s}_{n\%}$, where t is the time of incubation at an elevated temperature before protoplasts have reached a specified stage of fusion, s, in n% incidence (by interpolation). The reciprocal of $\underline{t}^{s}_{n\%}$, n being fixed, is thus considered a measure of the rate of the specified process. The choice of n within the range of 10-20% did not affect the following conclusion. The dependence of temperature of $\underline{t}^{f}_{15\%}$ for the fused-sphere stage, f, is shown in Fig. 2. The relation between the time of fusion and the absolute temperature, T, fits the equation,

$$1/\underline{t}^{f}_{15\%} = A_1 \exp(-\Delta E_1/RT)$$

where A_1 is constant to the system, R is the gas constant, and ΔE_1 is the activation energy of the fusion process. From the plot, ΔE_1 was calculated as 9.7 kcal/mol. Likewise, the relation between the time of fusion for the sum of both fused-sphere and face-adherent protoplasts, $\underline{t}^{m}_{20\%}$, fits the equation,

$$1/\underline{t}^{m}_{20\%} = A_2 \exp(-\Delta E_2/RT)$$

giving a ΔE_2 of about 15 kcal/mol.

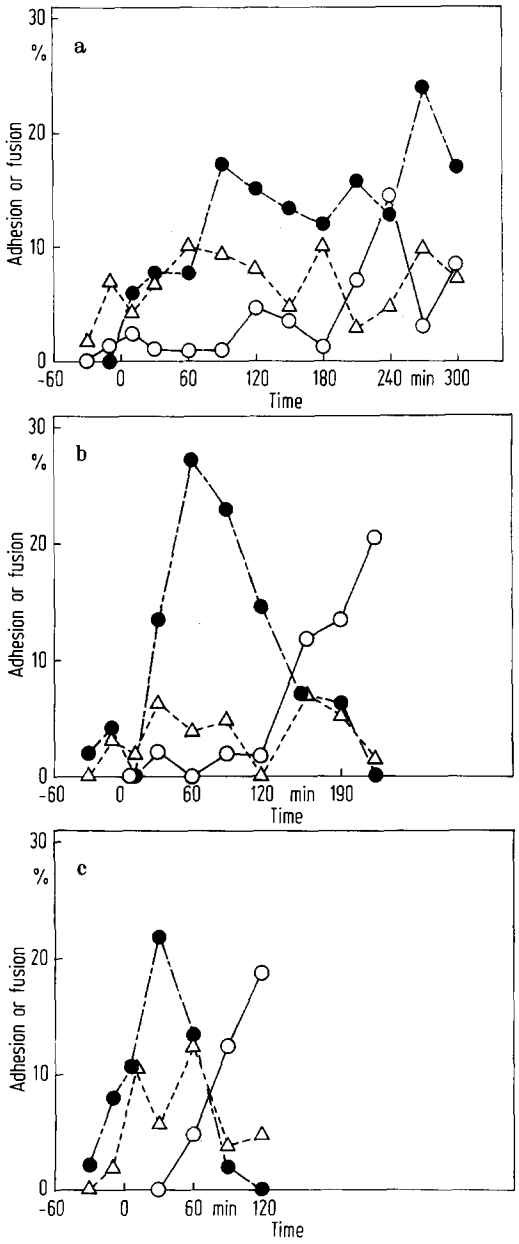

Fig. 1a-c. Plots of the extent of adhesion or fusion of protoplasts against incubation time in the presence of 40% (w/v) PEG and 10 mM $CaCl_2$ in 0.6 M sorbitol. The incubation temperature was changed at time = 0 from 2° ± 1°C to; a 2°, b 13°, and c 23°C
Δ = point-adherence stage; • = face-adherence stage;
○ = fused-sphere stage

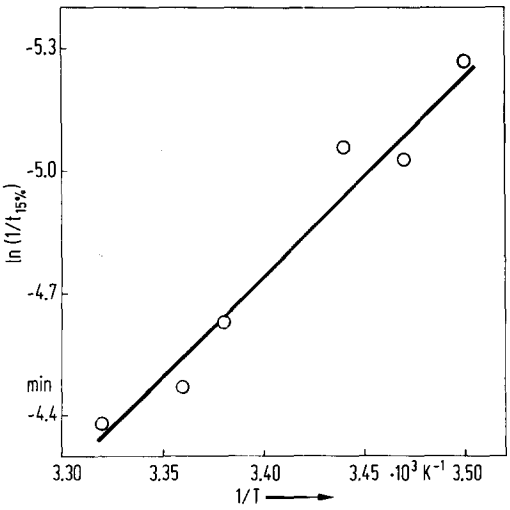

Fig. 2. Temperature dependence of the time of fusion of protoplasts in the presence of 40% (w/v) PEG and 10 mM $CaCl_2$ in 0.6 M sorbitol

Discussion

The values for the activation energy are compatible with the flow activation energy of liposomes and biological membranes, which is 15 to 7 kcal/mol, depending on the phospholipid composition (Shinitzky and Inbar 1976). The phospholipid compositions of plant protoplast membranes were reported in a previous paper (Yamada et al. 1979) and were discussed in relation to membrane fluidity and to the dynamic features of the membrane that are now believed to play a major role in the control mechanism that determines cell growth and differentiation as well as protoplast fusion. Unfortunately, no information on the membrane fluidity of plant protoplasts is available at present; therefore, a direct comparison between protoplast fusion and membrane fluidity is precluded. However, we believe that the present data on the activation energy of fusion provide evidence that membrane fluidity is important in regulating protoplast fusion.

The fusion of *R. serpentina* protoplasts was induced only in the presence of fusion-inducing agents, between 0° and 45°C with an incubation time of five hours at pH 5 to 9. Interestingly, the activation energy, ΔE_2, that represents the induction of fusion, differs from ΔE_1, which can be considered to represent approximately the subsequent spread of membrane fusion over the surface of the protoplasts to the fused-sphere stage. This suggests that the regulation factors in the former process are not identical with those of the latter. We also noted that 9% or 16% (w/v) PVA in 0.6 M sorbitol and 10 mM $CaCl_2$ induced fusion of protoplasts (Nagata 1978; Senda et al. 1978), but the rate of subsequent passage to the fused-sphere stage was low compared to that with PEG, although it increased with a rise in temperature. This rate also increased on replacement of the medium with a solution of lower osmotic pressure.

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