RCA₁-mediated agglutination and fluidity of *Mimosa pudica* chloroplast envelope membrane

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Abstract. The thermotropic phase transition temperature in the Chloroplast envelope membrane of *Mimosa pudica* has been measured using a fluorescent probe pyrene, for the first time. RCA₁-induced agglutination does not change the fluidity picture in general but reduces the fluidity uniformly throughout the whole range of temperature (15°–55°C). The turbidimetric assay of this agglutination reveals that this is less in case of membrane than in liposome, for which several explanations are discussed. Also, we have shown that divalent cations, like Ca²⁺ and Mg²⁺ do not affect the lectin-induced agglutination.

Keywords. *Mimosa pudica*; phase transition; chloroplast envelope membrane; RCA₁-induced agglutination.

1. Introduction

Because of its extreme sensitivity to various stimuli, the plant *Mimosa pudica* had been the subject of investigation since as early as 1926 (Bose 1926). We report here our study of the fluidity picture and the thermotropic phase transition within the chloroplast envelope membrane of *M. pudica* by using an excimer-forming fluorescent probe pyrene, a probe quite frequently used in the study of both model and biological membranes (Galla and Sackman 1974; Sengupta et al 1976; Flamm et al 1982; Nandy et al 1983). The landscape of the surfaces of the membrane and the liposome prepared from the polar lipid isolates therefrom, had been surveyed by assaying turbidimetrically the biospecific interaction of RCA₁, a lectin from *Ricinus communis*, with the sugar residues of the membrane glycoprotein and/or glycolipids (Rendi et al 1976). The effect of divalent cations Ca²⁺ and Mg²⁺ on this agglutination had been investigated and reversal of agglutination by addition of galactose had been performed. The lectin induced agglutination did not affect the qualitative nature of the membrane phase transition profile, except that there was a general decrease in the fluidity.

2. Materials and methods

RCA₁ was isolated from locally available *R. communis* (Nicolson and Lacorbiere 1973).

Chloroplast envelope membrane was separated from the fresh leaves of *M. pudica* (Poincelot 1973). Lipids were isolated (Bligh and Dyer 1959) and liposome was prepared by sonication of the polar lipid dispersion (Sengupta et al 1982) in PBS buffer.

Pyrene was incorporated within the membrane (0.3 mg pyrene per 1 ml of membrane suspension in 50 mM HEPES buffer at pH 7.2, containing 5 mg protein) for fluorescence study (Nandy et al 1983).
Agglutination induced by RCA₁ was assayed turbidimetrically by measuring the rate of change in absorbance value at 500 nm at 25°C in a Hilger spectrophotometer (Rendi et al 1976). Final concentration of lectin in the assay system was 70 μg/ml (buffer: HEPES for membrane suspension and PBS for liposome preparation).

In order to study the effect of divalent cations on the RCA₁-induced agglutination, the assay was carried out (Rendi et al 1979) in presence of 2 mM divalent cations Ca²⁺ (as in CaCl₂) and Mg²⁺ (as in MgCl₂). Reversal of agglutination was measured after addition of galactose (10 mM) to the assay mixture.

3. Results and discussion

The ratio of intensity peaks at 485 nm and 390 nm \( (I'/I) \) of the pyrene-probed membrane, when illuminated at 320 nm is a measure of the fluidity of the probe's environment (Galla and Sackman 1974) and the change in slope in \( I'/I \) versus temperature diagram indicates a phase transition (Flamm et al 1982) at that temperature.

In the chloroplast envelope membrane, a distinct phase transition is obtained around 20°C (figure 1A) showing that like most of the other biological membranes, this membrane is also fluid at the ambient temperature. ESR study reports a transition of phase in the chloroplast envelope membrane of other plants in the range 10°-30°C.

![Figure 1](image-url)  
*Figure 1. Thermotropic phase transition of chloroplast envelope membrane of Mimosa pudica. (a) ○, normal phase transition, (b) ●, in the presence of 70 μg/ml of RCA₁. Arrow indicates approximate phase transition temperature.*
whereas quite a few other techniques detect a major transition below 0°C (Quinn and Williams 1978). Due to technical limitations, the low temperature transition could not be observed here. In the turbidimetric assay of the interaction of RCA₁ with the membrane and the liposome prepared from its lipid isolates, it is observed that lectin agglutination is more in liposome compared to that in the membrane (81.8% compared to 21.8% figure 2). This leads us to the conclusion that more receptors are available on the liposome surface than that on the membrane (may also be due to the presence of more lipid molecules). In order to explain this enhanced agglutination, we suggest either one or both of the following possibilities:

(i) The glycolipids and the phosphatidylinositol contents of the chloroplast envelope membrane of *M. pudica* (Duttachoudhury and Chakrabarti 1980) are the possible lectin receptors. In liposomes the 'cryptic' binding sites of the receptor glycolipids are exposed, whereas these lectin-binding sites are probably shielded by the proteins, specially extrinsic, in membrane preparation (Gordon et al 1977).

(ii) In the membrane, proteins interact with endogenous glycosylated receptors forming 'self-neutralised' closed complex (Bowles 1979) which are disrupted in liposomes when proteins are removed from the system.

The study on the effect of divalent cations shows that addition of Ca^{2+} and Mg^{2+} ions does not change the RCA₁-mediated agglutination sufficiently (figure 2). The binding of the lectin in both the membrane and the liposome can be reversed by the
addition of galactose; because of the competition for binding taking place between the bound glycolipid and/or glycoprotein and the externally added galactose.

In a separate set of experiments, we have studied the effect of this RCA₁-induced agglutination on the fluidity profile of the envelope membrane. Addition of lectin has not changed the qualitative nature of the phase transition curve but there is a significant decrease in the \( I''/I \) value in the experimental range (figure 1B). This can be explained by anticipating proximity of the RCA₁ receptors in the membrane which allows more cross-bridge formation by RCA₁, imparting an over-all rigidity to the system. As a result, \( I''/I \) decreases indicating a less fluid environment.

Due to the similarity between the lectin-receptor interaction and antigen-antibody reaction, the observed results are of considerable biological significance.

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