Figure 1 ***a*** **and** ***b***. *Vertebraria indica* Royle, longitudinal sections of the specimens to show diagonally and vertically penetrating axes in the sediments, respectively. B S.I.P. Museum specimen numbers 36863 & 36864.

have been required for peat formation of Gondwana coals cannot be sufficiently provided by few *in situ* preserved plants and their root system. It is logical to think that root system already embedded in the palaeosols had better chances of getting preserved *in situ* than other plant parts as found in Ib river and other coalfields of Lower Gondwana of India.

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Role of phospholipase C in the cryptobiotic cysts of the fairy shrimp *Streptocephalus dichotomus*

As our earlier report[1] disclosed the occurrence and functional significance of the enzyme PC-PLC (phosphatidylcholine cholinephosphohydrolase) in the cryptobiotic cysts of the fairy shrimp *Streptocephalus dichotomus*, the involvement of biological catalysts (enzymes) in the hydration-dependent hatching of the cysts becomes quite evident.

Ever since the introduction of term 'cryptobiosis' in biology by Keilin[2], it has fascinated many investigators due to its unique features like maintenance of embryonic viability during thermal stress and the ability to withstand dehydration. *S. dichotomus*, which inhabits the freshwater ponds, undergoes cryptobiosis during dry summer period in order to overcome the adverse conditions Though a detailed study on the egg morphology[3], biochemistry[4] and reproductive biology[5]

has been carried out, the role of enzymes involved in signal transduction has not yet been investigated in detail.

Although PI-PLC (phosphatidylinositol phosphodiesterase) has been studied extensively in mammals and micro-organisms[6], reports on the role of its substrate, namely phosphoinositides, are available only in the embryonic development of sea urchins[7]. As a growing body of evidences disclose the significance of inositol phospholipids[8] and their hydrolytic products like DAG[8] in the transduction of signals from external to internal cellular system, the role of PI-PLC becomes seldom negligible.

As cyst hatching involves hydration for a definitive period (24 h), presumably some signal is transduced through messenger molecules. This, and the earlier results which revealed the role of calcium[9], $pH_{(i)}$ (ref. 10) and PC-PLC[1] initiated studies on whether or not the PI-PLC has any role in cyst hatching. Cysts were collected from the animals as described previously[1], homogenized in Tris–acetate buffer (0.01 m, pH 7.5) and centrifuged at $4500 \times g$ for 20 min in a KUBOTA-1500 refrigerated centrifuge. The supernatant was decanted and the pellet was redissolved in the same buffer and centrifuged as above. Both the supernatants were pooled up and used as the sample for the assay.

Activity assay of PI-PLC was carried out by the alkaline phosphatase releasing method as described by Ikezawa and Taguchi[11]. In brief, male rat (Albino, Wistar strain) kidneys were excised and homogenized in ice-cold 0.25 M sucrose solution. To the homogenate 0.5 ml of the sample was added and incubated at 37°C for 30 min. After the incubation, 1 ml aliquot was withdrawn, cooled in an ice bath and then centrifuged at $1,09,000 \times g$ for 90 min in a Beckman L7-65 ultracentrifuge. The supernatant represented the cytosolic fraction and the sediment the particulate fraction. Both cytosolic and particulate fractions were assayed for the alkaline phosphatase activity according to Engstrom[12]. The absorbance was recorded at UV–visible 160 Å spectrophotometer.

Figure 1 depicts the activity assay results of PI-PLC at different hours of hydration of the cysts of *S. dichotomus*. The enzyme activity of the cytosolic fraction increased in the first 12 h of hydration (0–12 h), followed by a subsequent decrease in the next 12 h (12–24 h) of hydration, whereas in the particulate fraction the activity of the enzyme decreased initially (0–12 h) and increased in the next 12 h (12–24 h) of hydration. Although in both cytosolic and particulate fractions the activity of the enzyme varied considerably between the first 12 h (0–12 h) and the next 12 h (12–24 h), a unique feature observed is that the activity in cytosolic fraction is inversely proportional to particulate fraction. In other words, whenever there is an increase/decrease in the PI-PLC activity of the cytosolic fraction, it is inversely proportional to the particulate fraction.

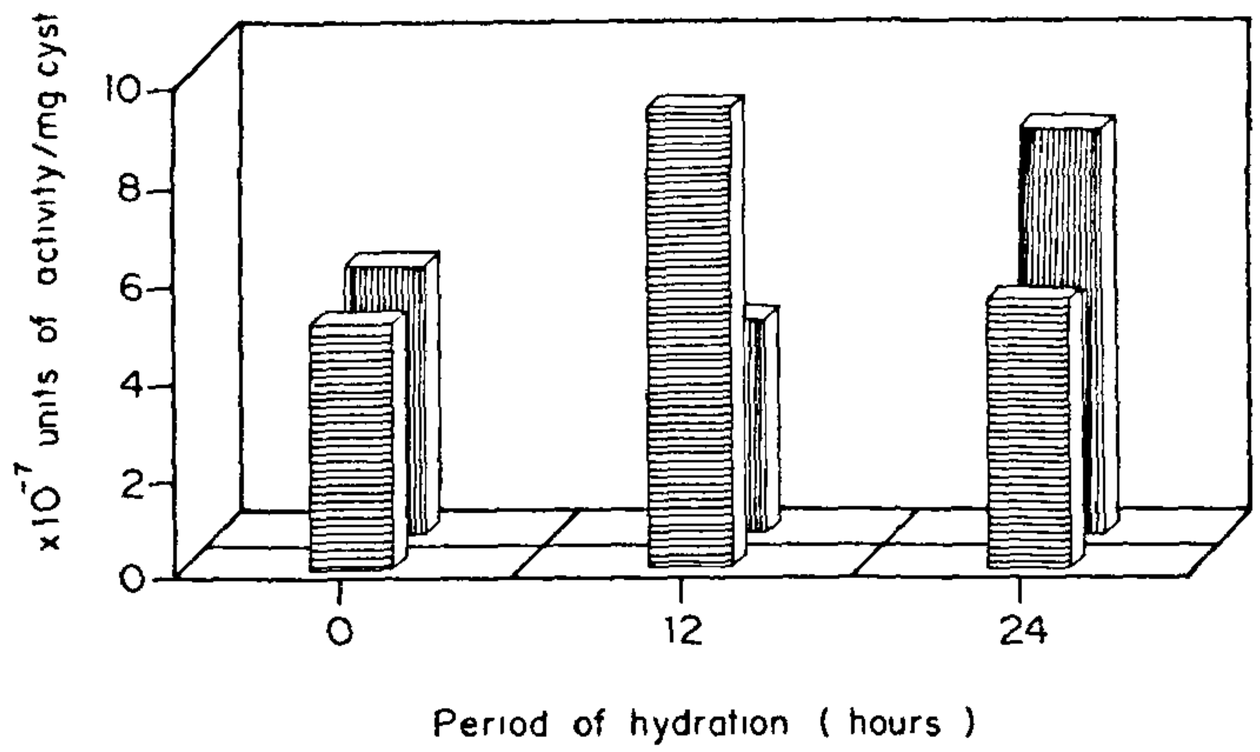

Figure 1. PI-PLC activity in the cryptobiotic cysts of the fairy shrimp Streptocephalus dichotomus. ▤, Cytosolic fraction; ▥, Particulate fraction.

Unlike PC-PLC, where there is a steady-state increase in the activity up to the period of hatching[1] (i.e. 24 h), PI-PLC has a fluctuating trend. As PI-PLC is a membrane-bound enzyme, it is presumable that the activity is high in the particulate fraction than in the cytosolic fraction at 0 h of hydration. The increase in activity in the cytosolic fraction at 12 h of hydration can presumably be accounted for its transmembrane mobility as it has to hydrolyse the cytosolic IP. Similarly, the decrease in activity in the cytosol at 12–24 h of hydration implies the culmination of PI-PLC-catalysed reaction.

From the present report it is evident that a change (increase/decrease) in the activity of PI-PLC does occur in both cytosolic and particulate fractions pertaining to the period of hydration.

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