論　　説

ソテツ花粉の表在性プロテアーゼについて

原　　彰・松ヶ野一郷・小林　昭*

Protease Associated with the Cell Surface of Cycad Pollens

Akira HARA, Kazusato MATUGANO & Akira KOBAYASHI*

It has been reported by many workers that proteins including various enzymes and other substances were diffused from the intact pollen grains[1-13], and that these materials were probably participated in the pollen germination, tube nutrition and incompatibility reactions[3,4,10]. In case of the pollens of cycad, *Cycas revoluta* THUNB., ribonuclease, phosphomonoesterase, and ultraviolet ray-absorbing materials were eluted with salt solutions from the cell-surface[6]. Invertase was found to exist at the cell wall-site by forming a stable complex with pectic substances[8]. In this paper, the results of release and readsorption study of protease associated with the surface of the cycad pollens are described.

Matured pollen grains were obtained from the male strobiles gathered in the naturally growing cycad groves at Mageshima, Nishino-Omote, Kagoshima, on June 1973, and were stored at below −20°C. Pollen cells were preliminarily suspended in water and washed twice by centrifugation in order to remove extra ultraviolet ray-absorbing materials and trace ribonuclease, phosphomonoesterase and protease. The activities of ribonuclease, phosphomonoesterrase, invertase and alkaline pyrophosphatase were measured by the methods reported previously[6-8]. Protease was assayed in a following way by using a commercial preparation of bovine hemoglobin (Miles Laboratories, Inc.) as the substrate. One ml of reaction mixture containing 0.25% of hemoglobin, 0.05 M acetate buffer, pH 4.0, and an appropriate amount of enzyme was incubated at 37°C for 15 min. The reaction was stopped by adding 0.5 ml of 0.6 M trichloroacetic acid, and after standing for 10 min the mixture was centrifuged. The yielded proteolytic products in 0.5 ml of the supernatant was determined by the modified Lowry's method[14] which gave a linear photometric responce. One unit of protease was arbitrarily defined as the amount of enzyme which produced a color equivalent to 1 μg tyrosine per min under the indicated conditions.

As shown in Table 1, protease, ribonuclease and phosphomonoesterase were scarcely released from the pollen cells with 0.05 M acetate, pH 5.0, and with the same buffer added with 0.1 M EDTA or 0.5 M sucrose, however, released fairly well with 0.5 M NaCl-added acetate at pH 5.0 or with 0.05 M Tris-HCl

* （〒890　鹿児島市郡元1丁目21番24号　鹿児島大学農学部　生物化学及栄養化学研究室）

* Laboratory of Biochemistry and Nutritional Chemistry, Faculty of Agriculture, Kagoshima University, Kagoshima, Japan

Table 1. Effect of various buffer solutions on release of enzymes from pollen cells

Buffer	Activity of enzyme (unit/10 ml)									
	Protease		Alkaline pyrophosphatase		Ribonuclease		Phosphomono esterase		Invertase	
	sup	ppt	sup	ppt	sup	ppt	sup	ppt	sup	ppt
0.05 M acetate, pH 5.0	31	403	0.03	0.20	0.75	4.10	0.07	0.78	0.1	16.9
0.1 M EDTA in 0.05 M acetate, pH 5.0	17	416	0.02	0.22	0.50	3.85	0.07	0.78	0.1	16.6
0.5 M sucrose in 0.05 M acetate, pH 5.0	22	426	0.01	0.29	0.55	4.05	0.07	0.82	0	14.8
0.5 M Nacl in 0.05 M acetate, pH 5.0	1128	190	0.11	0.13	5.30	2.55	0.74	0.47	0.2	17.9
0.05 M Tris-HCl, pH 8.0	1102	148	0.06	0.15	6.45	2.05	0.29	0.73	0.3	17.1
Whole cell activity	1762		58.13		9.75		4.50		107.5	

A mixture of 10 ml each of 10% pollen cells-suspension and the indicated buffer solution was stirred for 30 min in an ice bath and then centrifuged. The supernatant fluid together with washings (sup) and the precipitates suspended in water (ppt) were made volume to 10 ml, respectively, and assayed for the enzyme activities. Whole cell activity was assayed on a suspension of cells disintegrated in a teflon-glass homogenizer for 10 min at pH 7.0.

at pH 8.0. The activity of released protease came up to about 60% of the whole cell activity. Alkaline pyrophosphatase, proved previously to be a cell-interior enzyme[7], was detected in neither the supernatant nor the precipitate fraction, but only in the disintegrated pollen cell-suspension (the whole cell activity) (Table 1). This result indicated that the inner contents of the cells were not eluted under these conditions, therefore, the released enzymes were assumed to be existed originally in the surface region of the pollen cells. The behaviors of invertase were quite different from those of other cell-surface enzymes; its activity was definitely found in the precipitate and was not eluted with NaCl or an alkaline buffer (Table 1). A successful release with NaCl was attained on the cell-wall fraction obtained after disintegration of the pollen cells[7].

The results in Fig. 1 showed that the release of protease depended upon pH and reached to the maximum level at pH ranges above 7.0. No significant release of the enzyme was observed at pH 4.0. The apparent activity of released enzyme in the supernatant at pH 7.0 amounted about 2.5-fold of the precipitate at pH 4.0.

A readsorption experiment of protease to the pollen cells was made with aliquots of partially purified

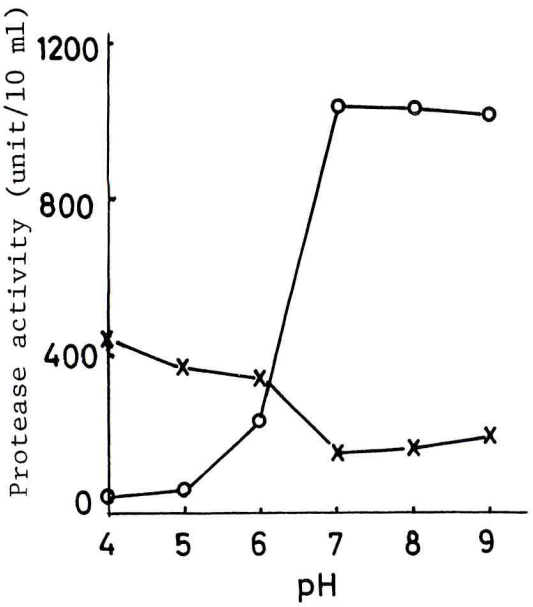

Fig. 1. Effect of pH on release of protease from pollen cells.
The pollen cells were suspended in water, 10%, 5 ml of which was added with 5 ml of 0.1 M buffer solution (acetate for pH 4-6, Tris-HCl for pH 7-9). Each mixture was stirred for 30 min in an ice bath and then centrifuged. The enzyme activity in the supernatant (o—o) and the precipitate (x—x) was assayed as described in the legend of Table 1.

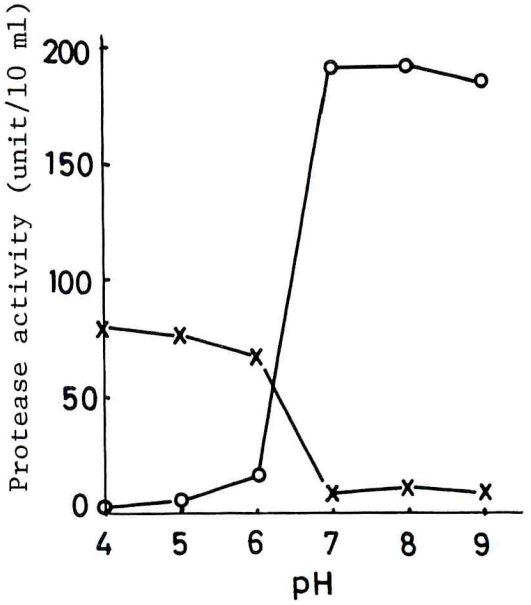

Fig. 2. Effect of pH on readsorption of purified protease to pollen cells.
The pollen cells, 100 mg, were suspended in 4.5 ml of 0.05 M buffer (acetate for pH 4-6, Tris-HCl for pH 7-9) and heated at 100°C for 10 min to inactivate the protease. The suspension was added with 0.5 ml solution of the purified protease (190 units), stood for 30 min with occasional stirring, and centrifuged. The enzyme activity in the supernatant (o—o) and the precipitate (x—x) was assayed as described in the legend of Table 1.

preparation (total activity, 22, 800 units ; protein, OD_{280} = 9.22). It was obtained by successive chromatogrpahies and gel-filtration with DEAE-Sephadex A-50, SP-Sephadex C-50 and Sephadex G-100 of crude protease (total activity, 104,000 units ; protein, OD_{280} = 416) which was extracted with 0.05 M Tris-HCl buffer, pH 8.0, from 40 g fresh weight of the cells. As shown in Fig. 2, the enzyme was almost completely adsorbed to the pollen cells in acidic, but not alkaline, pH range tested. The apparent activity of the adsorbed enzyme at pH 4-6 remained in below 40% of that of the added enzyme. These results coincided entirely with those of the release experiments (Fig. 1) and suggested that release and readsorption of the enzyme were quite reversible processes. The low activities observed on the adsorbed enzyme (Fig. 1 and 2, at pH 4-6) were presumably due to a possible masking or some structural changes of the enzyme protein caused by the binding to the cell-surface.

Unequivocally the binding of enzymes to a cell-surface must be regulated by the characteristics of enzyme proteins and binding substances of the cell-surface. It is likely from the pH dependence and the reversibility of the release of protease that the binding of the enzyme to the pollen cell-surface is effected by ionic linkages. Readsorption of ribonuclease to the cell-surface[7] resembles that of protease (here obsersed), though invertase is, in a different way, completely adsorbed to the cell wall in the range of

pH 4-9[7]. The authors showed already that invertase formed a complex with pectic substances prepared from the cell wall of the cycad pollen, and consequently its activity was stabilized[8].

There seems to be at least two types of reaction between the enzyme and the cell-surface ; one is represented by the behaviors of protease and another, of invertase[7,8]. These facts will conceivably offer some models for elucidating enzyme-cell-surface interrelationships. Furthermore, it will be of interesting to study on the behaviors of the enzymes associated to the pollen cell-surface during the pollination and germination processes.

REFERENCES

(1) B. M. Johri and I. K. Vasil : Botan. Rev., 27, 326 (1961)

(2) T. P. Petrovskaya-Baranova and N. Tsinger : Botan. Zh., 47, 1327 (1962)

(3) R. G. Stanley and H. F. Linskens : Physiol. Plant., 18, 47 (1965)

(4) Y. Makinen and J. L. Brewbaker : ibid., 20, 477 (1967)

(5) Y. Makinen and T. MacDonald: ibid., 21, 477 (1968)

(6) A. Hara, K. Yoshihara and T. Watanabe : Nippon Nogeikagaku Kaishi, 44, 385 (1970)

(7) A. Hara, M. Yamamoto, Y. Horita and T. Watanabe : Mem. Fac. Agr. Kagoshima Univ., 8 No. 2, 27 (1972)

(8) A. Hara, A. Sakamoto and A. Kobayashi : Agr. Biol. Chem., 38, 2287 (1974)

(9) R. B. Knox, J. Heslop-Harrison : Nature, 223, 92 (1969)

(10) R. B. Knox, J. Heslop-Harrison and C. Reed : ibid., 225, 1066 (1970)

(11) R. B. Knox and J. Heslop-Harrison : J. Cell Sci., 6, 1 (1970)

(12) J. Heslop-Harrison, Y. Heslop-Harrison, R. B. Knox and B. Howlett : Ann. Bot., 37, 403 (1973)

(13) R. B. Knox : J. Cell Sci., 12, 421 (1973)

(14) E. F. Hartree : Anal. Biochem., 48, 422 (1972)