various parts of the country, as symptoms of SPMMV infection have also been observed in southern India (A. Varma, unpublished findings). It is possible that the virus diseases in sweet potato may pose greater problem than realized so far.

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Resolution of peripheral membrane proteases from mitochondrial pellet of rat submaxillary gland

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Peripheral membrane protease from mitochondrial pellet of rat submaxillary gland was resolved into three components on a DEAE-cellulose column. These enzymes were found to be serine proteases. They showed differences in their responses to several protease inhibitors, in their pH optima and in their thermostability.

MEMBRANE-BOUND intracellular proteases are increasingly being viewed as important in intracellular membrane protein catabolism and membrane traffic regulation[1]. Compared to studies on the soluble counterparts, studies on membrane proteases are much fewer. Despite several investigations on a large number of soluble proteases[2,3], work on intracellular membrane proteases from rat submaxillary gland has not been reported. We have observed that mitochondrial pellet from rat submaxillary gland contains significant protease activity. The present communication reports the presence of three forms of serine proteases in KCl extract of mitochondrial pellet from rat submaxillary gland.

A 10% (w/v) homogenate of pooled glands from albino rats (120–160 g) of either sex was prepared in 0.01 M sodium phosphate buffer (pH 7.4) containing 0.25 M sucrose. The homogenate was centrifuged at 800 *g* for 10 min and the resuspended pellet was centrifuged again. The pooled supernatant was centrifuged at 12,000 *g* for 20 min. The pellet, washed twice with the same buffer, was suspended in 0.01 M phosphate buffer (pH 7.4) containing 1 M KCl and kept at 4°C for 3 h with occasional stirring, and centrifuged at 105,000 *g* for 1 h. The step was repeated. KCl-extracted pooled supernatant was then ammonium-sulphate fractionated (40–80% saturation), the ammonium-sulphate precipitate *dissolved in 0.05 M Tris–HCl buffer (pH 8.0) and the* solution dialysed overnight against the same buffer.

The dialysed material was passed through a DEAE-cellulose column pre-equilibrated with the dialysis buffer. The column was eluted initially with the dialysis buffer, then a linear gradient between 0.05 M and 0.5 M Tris–HCl (pH 8.0), and finally with 0.5 M Tris–HCl (pH 8.0) containing 0.5 M KCl. All steps were carried out in the cold.

Protease activity was assayed at pH 8.5 by the method of Erlanger *et al.*[4] using α-*N*-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) as substrate, except that the activities of column eluates were monitored by the method of Schewart and Takenaka[5] using α-*N*-benzoyl-L-arginine ethyl ester (BAEE) at pH 8.5.

The protease activity was resolved into three

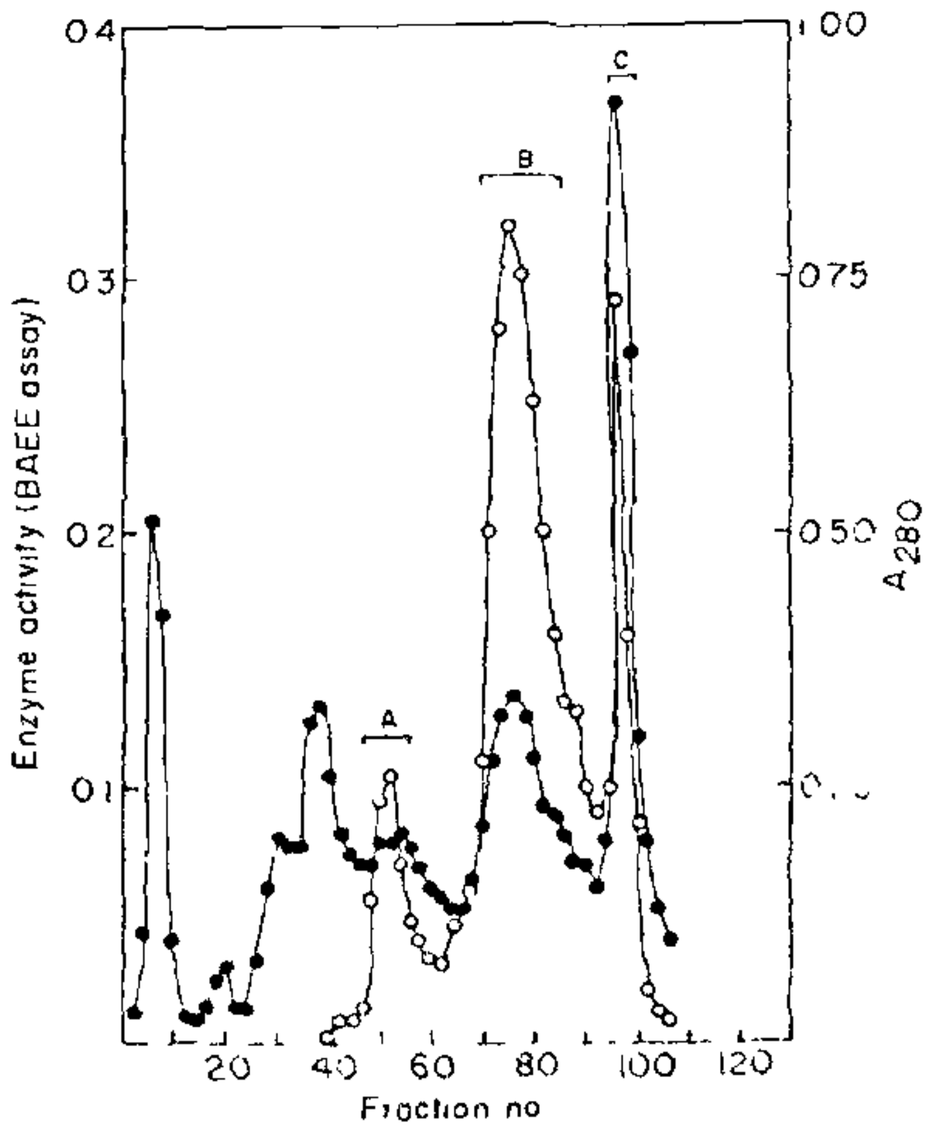

Figure 1. DEAE-cellulose chromatography. Fraction volume, 1.4 ml. ——●——, Absorbance at 280 nm, - - - - -, protease activity (10 µl fraction). Details of elution are given in the text. A linear gradient between 0.05 M and 0.5 M Tris–HCl (pH 8.0) was applied (fractions 16–88).

components on the DEAE column (Figure 1). The first two components (A and B) were eluted during gradient run and the third one (C) was eluted after the gradient run.

Table 1 describes the differential effects of several inhibitors on these enzyme fractions. Soybean trypsin inhibitor (SBTI) inhibited the enzyme activity of fractions A and C strongly but had no significant effect on that of fraction B. Lack of significant inhibition by *N*-ethylmaleimide and *p*-chloromercurybenzoate (pCMB) suggests that neither enzyme contained active SH group. Dithiothreitol (DTT) inhibited only the activity of fraction A, which indicates that the enzyme might require the presence of an intact –S–S– bond for its catalytic activity. Though phenylmethylsulphonyl fluoride (PMSF) failed to produce significant effect on any of the fractions, other serine-protease inhibitors, like antipain, benzamidine and aprotinin, inhibited the enzyme activity of all fractions, suggesting that the enzymes are serine proteases. Differential inhibition was observed with aprotinin at 5 KIU (kallikrein inhibitor unit), but higher doses inhibited all enzymes strongly. *N*-α-*p*-Tosyl-L-lysine chloromethyl ketone (TLCK), a strong trypsin inhibitor, did not inhibit fractions A and B while C was only 24% inhibited.

The enzymes showed differences in their pH optima and thermostability. It was observed that the enzyme activity of A showed pH optimum at 8.0 whereas enzymes B and C showed maximum activity at pH 9.0 (data not shown). Figure 2 shows that enzyme activity of none of the fractions was altered significantly when heated up to 50°C. But at 60°C the enzyme activity of A was reduced by 79% and that of C was reduced by 34%; the enzyme activity of C was reduced to a greater extent at 70°C. The activity of B was not inhibited significantly up to 70°C, suggesting that the enzyme is thermostable.

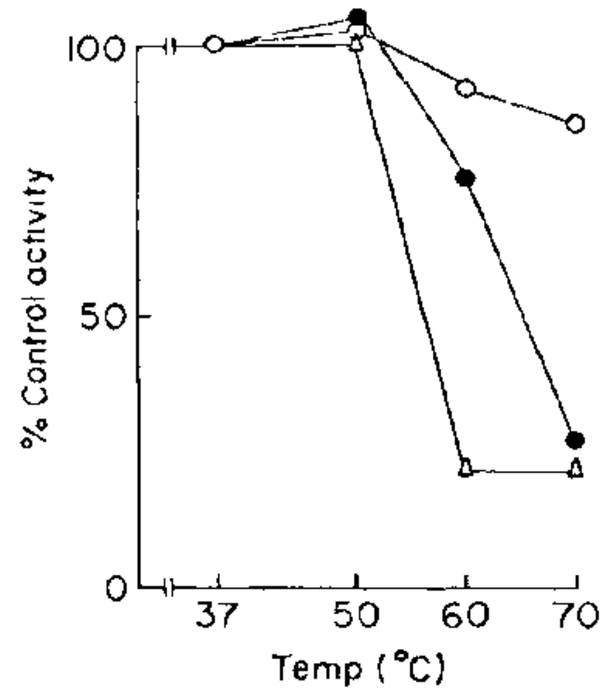

Figure 2. Thermostability curves. An aliquot of each enzyme in 50 mM Tris–HCl (pH 8.0) was heated at different temperatures for 10 min. Protease fractions: A (—△—), B (—○—), C (—●—).

The mitochondria preparation was 72% pure as evidenced by succinic dehydrogenase activity. Activity of the three proteases in the alkaline pH range suggests that the enzymes are not from any lysosomal residue in the pellet. Release of the proteases from mitochondrial membranes by high salt concentration establishes their probable nature as peripheral-membrane proteins. Our data indicate the presence of three forms of proteases in KCl extract of mitochondrial pellet from rat submaxillary gland.

Table 1. Effect of various enzyme inhibitors on fractions A, B and C of rat submaxillary gland mitochondrial membrane proteases.

Inhibitor	% Inhibition		
	A	B	C
SBTI (100 μg)	96.4	14.0	93.0
(200 μg)	97.4	20.0	96.0
LBTI (100 μg)	32.0	0.0	34.7
DTT (1 mM)	44.3	0.0	4.0
N-ethylmaleimide (1 mM)	0.0	2.0	12.4
pCMB (1 mM)	3.0	0.0	15.0
Antipain (50 μg)	83.3	42.4	86.6
PMSF (1 mM)	10.0	6.0	41.8
Benzamidine (50 μg)	58.8	51.6	67.1
Aprotinin (5 KIU)	92.1	16.3	48.7
(10 KIU)	94.6	50.6	67.2
(50 KIU)	97.0	89.6	91.2
Pepstatin A (50 μg)	3.0	0.0	27.0
Bacitracin (50 μg)	44.4	2.0	21.6
TLCK (1 mM)	0.0	0.0	24.0

An aliquot of each enzyme fraction was preincubated for 10 min at room temperature with each of the inhibitors.

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