very common in the deep sea (*19, 21*). The continuous reproducers, in particular, can be considered typical deep-sea species. The bivalves examined have not been recorded from depths shallower than the San Diego Trough and the polychaete belongs to a family which is almost exclusively deep-sea, *Fauveliopsis glabra* being the shallowest recorded species (*22*). Thus, the available data suggest that year-round reproduction is the common pattern in the deep-sea benthos.

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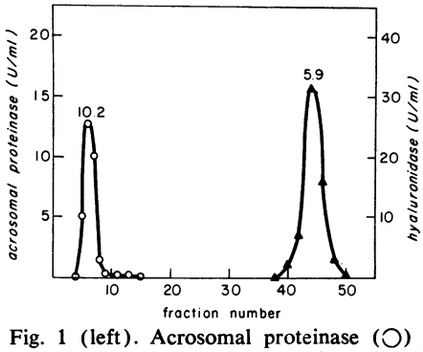
Amino Acid Content of Rabbit Acrosomal Proteinase and Its Similarity to Human Trypsin

Abstract. *Rabbit acrosomal proteinase from epididymal spermatozoa of 44 male rabbits was purified by subcellular fractionation, sucrose density gradient centrifugation, and electrofocusing; the specific activity of the purified product was 20,047 units per milligram, a value similar to that observed for pancreatic trypsins from various sources. The molecular weight determined from the amino acid analyses and ultracentrifugation was about 22,000. This rabbit acrosomal proteinase showed great similarity to pancreatic trypsin, especially to human pancreatic trypsin, both in the number of individual amino acids and in the total number of residues. This similarity was confirmed by an antigenic cross reaction between rabbit antiserum to bovine pancreatic trypsin with human, rabbit, rhesus monkey, and bull acrosomal proteinase.*

Acrosomal proteinase is present in the acrosomes of spermatozoa from numerous species, and its proteolytic activity is essential to fertilization (*1, 2*). Its enzymic properties are similar to those of pancreatic trypsin (*1–7*), and evidence for a trypsinogen-like precursor has also been reported (*8*). However, molecular weight estimations for this enzyme have ranged from 27,300 to 59,000, values considerably higher than those reported for pancreatic trypsin (*2, 4, 5, 7*). It was apparent that an amino acid analysis might help to explain some of the similarities and differences between pancreatic trypsin and acrosomal proteinase, and this was the object of our study.

Rabbit (New Zealand White) epididymal spermatozoa were collected and fractionated subcellularly by means of sonication and sucrose density gradient centrifugation (*2*). The isolated sperm heads were extracted at 37°C with 0.2 percent Hyamine 2389 for 90 minutes, and the extract was centrifuged. This supernatant was fractionated on sucrose gradients at 216,000*g* for 17 hours at 5°C (*2*), and 87 percent of the enzyme was recovered in this step. The fractions containing both hyaluronidase and acrosomal proteinase in the region of 4.3*S* proteins were collected, dialyzed, and subjected to electrofocusing in a sucrose gradient (*p*H 7–10; 2 percent Ampholine, carrier ampholytes) for 70 hours at 3°C (LKB 8100 apparatus), a procedure providing good resolution of acrosomal proteinase (isoelectric *p*H, 10.2) and hyaluronidase (isoelectric *p*H, 5.9) (Fig. 1). The peak fractions of acrosomal proteinase collected from this column had a specific activity of 20,047 unit/mg, and this value represents a 118-fold purification of the enzyme extracted from whole, washed epididymal sperm prior to subcellular fractionation. No further increase in specific activity was obtained after a second electrofocusing.

This specific activity is similar to that observed for pancreatic trypsins from various sources, and similar specific activities were observed in three separate runs by this purification procedure. When the molecular weight of this hyaluronidase-free proteinase was determined by ultracentrifugation in sucrose gradients containing 2 percent acetic acid to prevent aggregation of the enzyme, the proteinase sedimented precisely with pancreatic trypsin in the region of 2.7*S* proteins. The peak fractions from electrofocusing were used



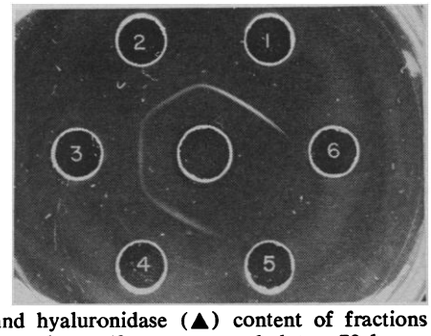


Fig. 1 (left). Acrosomal proteinase (○) and hyaluronidase (▲) content of fractions collected from the LKB 8100 electrofocusing column (2 percent ampholytes, 70 hours, 1000 volts, 3°C). Fig. 2 (right). Immunodiffusion plate with rabbit antiserum to bovine trypsin in the center well. The other wells contained (1) bovine trypsin, (2) human acrosomal proteinase, (3) bull acrosomal proteinase, (4) rabbit acrosomal proteinase, (5) human thrombin, and (6) bovine chymotrypsin.

for amino acid analyses on a Beckman-Spinco model 120C analyzer.

On the basis of the minimum molecular weight from the amino acid analysis and the molecular weight of the purified enzyme determined by ultracentrifugation in acidic sucrose gradients, we calculated the amino acid composition of acrosomal proteinase (Table 1). The results demonstrated great similarity in amino acid composition between rabbit acrosomal proteinase and the pancreatic trypsins, but especially with human trypsin. The glycine content was identical in both enzymes, while the structurally similar amino acid pairs lysine and arginine, threonine and serine, and tyrosine and phenylalanine were also present in identical numbers of residues when taken in pairs. That is, acrosomal proteinase has one less lysine, but one more arginine; it has two less serines, but two more threonines; and it has two less tyrosines, but two more phenylalanines. The other amino acids all differ by only one residue, with the exception of glutamic acid, valine, and isoleucine. Acrosomal proteinase has six less valine and four less isoleucine residues, but six more glutamic acid or glutamine residues when compared to human trypsin. Because of the small quantities of enzyme available to work with, we have not yet obtained an accurate determination of half-cystine and tryptophan.

Unfortunately, the amino acid composition of rabbit trypsin has never, to our knowledge, been reported; and the pancreatic trypsins analyzed for their amino acid content are from widely divergent species. However, we did compare the antigenic properties of these enzymes by preparing, with the use of Freund's adjuvant, an antiserum to crystalline bovine pancreatic trypsin since it is apparent from Table 1 that acrosomal proteinase is similar to bovine pancreatic trypsin as well as to human trypsin. To accomplish immunodiffusion with acrosomal proteinase the gel must be buffered at *p*H 5.0. At higher *p*H acrosomal proteinase forms dimers and larger aggregates within the well of the immunodiffusion plate, and the enzyme never diffuses into the gel in sufficient quantities to form precipitation bands. At *p*H 5.0, distinct cross reactions were obtained between rabbit antiserum to bovine pancreatic trypsin with human, bull, rhesus monkey, and rabbit acrosomal proteinases, confirming the structural similarities between acrosomal proteinase and trypsin (Fig. 2).

The similarity between acrosomal proteinase and trypsin is remarkable considering their widely different physiological roles, and it will be interesting to determine how similar the structures of these two enzymes are in the same species. It seems that this loss of some hydrophobic amino acid residues might allow a partial unfolding of the polypeptide chain, giving rise to the tendency of acrosomal proteinase to reversibly form dimers and larger aggregates at low *p*H, a phenomenon that we have observed many times in our laboratory when sucrose density gradient centrifugation was used for molecular weight determinations both at high and low *p*H. Whether any aggregation occurs between acrosomal proteinase molecules and other enzymes in vivo remains to be determined; but the enzyme does appear to be bound to the acrosomal membrane (*6*, *9*).

Table 1. Amino acid composition (number of residues) of rabbit acrosomal proteinase, and of human (*10*) and bovine (*11*) trypsin; N.D., not determined.

Amino acid	Acrosomal proteinase	Human trypsin	Bovine trypsin
Glycine	20	20	25
Lysine	10	11	14
Arginine	7	6	2
Threonine	12	10	10
Serine	22	24	33
Tyrosine	5	7	10
Phenylalanine	6	4	3
Histidine	4	3	3
Asx*	22	21	22
Proline	8	9	9
Alanine	14	13	14
Methionine	2	1	2
Leucine	13	12	14
Glx†	27	21	14
Valine	10	16	17
Isoleucine	8	12	15
Total	190	190	207
Half-cystine	ND	8	12
Tryptophan	ND	3	4

* Aspartic acid or asparagine. † Glutamic acid or glutamine.

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Heat Production and Temperature Regulation in Eastern Skunk Cabbage

Abstract. *The spadix of* Symplocarpus foetidus *L. maintains an internal temperature 15° to 35°C above ambient air temperatures of −15° to +15°C. For at least 14 days it consumes oxygen at a rate comparable to that of homeothermic animals of equivalent size. Temperature regulation is accomplished by variations in respiratory rate.*

The inflorescence of eastern skunk cabbage (*Symplocarpus foetidus* L.) weighing 2 to 9 g maintains a temperature 15° to 35°C above air temperature for a period of at least 2 weeks during February and March when air temperatures are from −15° to +15°C. The tissues of the inflorescence (spadix) are not frost-resistant and escape freezing by maintaining a high respiratory rate. An elevated respiratory rate [which Buggeln *et al.* (*1*) refer to as respiratory climacteric] in the inflorescences of members of the Arum family (Araceae) is common, but maintenance of that elevated respi-