Trypsin and a Trypsin-Like Enzyme from the Stomachless Cunner. Catalytic and Other Physical Characteristics

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A 24 000-Da protease isolated from the pancreas of cunner was classified as trypsin on the basis of its molecular size, ability to hydrolyze synthetic substrates N^{α}-benzoylarginine-*p*-nitroanilide (BAPA) and tosylarginine methyl ester (TAME), and inhibition by phenylmethylsulfonyl fluoride (PMSF), soybean trypsin inhibitor (SBTI), and benzamidine, as well as amino acid composition. A 14 000-Da protease from cunner pancreas was classified as trypsin-like on the basis of its molecular weight, ability to hydrolyze BAPA and TAME, inhibition by PMSF, SBTI, but not benzamidine, and amino acid composition different from other trypsins. Both enzymes possessed unusual heat stability, retaining more than 50% activity after 30 min at 100 °C, but differed in their stability at 25 °C when the pH was acidic or alkaline. For trypsin hydrolysis of BAPA, the Arrhenius energy of activation (E_a) was 1791 J/mol and at 25 °C K_m' was 0.73 mM and turnover number was 181 BAPA units/μmol of enzyme; and for trypsin-like hydrolysis of BAPA, E_a was 2054 J/mol, K_m' was 1.38 mM, and turnover number was 164 BAPA units/μmol of enzyme.

Even though studies on the physiological aspects of digestion in fish were initiated in the late 18th century (Spallazani, 1783) and early 19th century (Tiedman and Gmelin, 1827), there is a paucity of information on the different digestive enzymes of fishes. It is known that certain fish, such as Cyprinidae, Labridae, Gobiidae and Teradontidae, lack a distinct stomach (Ishida, 1936). The stomach aids protein digestion in several ways, notably by its secretion of acid proteases that denature and initiate hydrolysis of proteins. Previous studies (Jany, 1976; Pfleiderer et al., 1967; Mihalyi, 1978) indicate that trypsins and chymotrypsins from higher vertebrates do not efficiently hydrolyze native proteins, and it is generally assumed that acid denaturation and hydrolysis of food protein in the stomach is a necessary prelude to efficient protein hydrolysis by intestinal enzymes. In stomachless fish, the question arises whether digestion of protein in the absence of acid denaturation and gastric proteases is compensated for by (a) intestinal enzymes that are more efficient in digesting native proteins, (b) the secretion of greater quantities of intestinal digestive enzymes, or (c) such fish ingesting lesser amounts of food at any one time, thus permitting the digesting enzymes to mix more thoroughly with the food and thereby enhancing their action. According to Beauvalet (1933) intestinal secretions from stomached fish are not adequate in themselves to digest protein, whereas intestinal secretions form stomachless fish are sufficient to facilitate complete protein digestion. This observation is consistent with the view that such fish secrete enzymes more efficiently in catalyzing hydrolysis of native proteins. The hypothesis is further strengthened by the demonstration that a trypsin-like enzyme from stomachless crayfish hydrolyzed native ribonuclease and rapidly inactivated native lactate dehydrogenase, unlike trypsin from beef, an animal with a functional stomach (Pfleiderer et al., 1967). Hypothesis (b) is supported by the finding that there is a correlation between the concentration of digestive enzymes in the digestive system and the feeding activity in certain marine fish including the stomachless puffer (Chesley, 1934).

Trypsin and a trypsin-like enzyme were recently isolated from the pancreas of the stomachless cunner and demonstrated to be homogeneous on polyacrylamide gels (Simpson and Haard, 1985). Their molecular weights were determined and reported as 24 000 and 14 000 from trypsin and the trypsin-like enzyme, respectively. This study describes some of the properties of the two enzymes and their capacity to digest native proteins.

MATERIALS AND METHODS

Materials. Cunner (*Tautogolabrus adspersus*) were caught by scuba divers near St. Philips, Newfoundland, in the months of June and July and held in a large tank fed directly with ocean sea water at the Marine Sciences Research Laboratory in St. John's, Canada. They were transferred from the large tank in batches of 15 to a smaller aquarium that was maintained at 7 °C and fed chopped capelin (*Mallotus villosus*) at 2-day intervals for at least 2 weeks prior to sacrifice and removal of the pancreas for the study. They were not fed for the last 2 days before they were sacrificed.

N^{α}-Benzoylarginine-*p*-nitroanilide (BAPA), tosylarginine methyl ester (TAME), bovine hemoglobin (type II), ribonuclease from bovine pancreas (RNase, type I-AS), bovine pancreas trypsin (type III), ribonucleic acid from torula yeast (type II-S), *p*-chloromercuribenzoate (PCMB), phenylmethylsulfonyl fluoride (PMSF), soybean trypsin inhibitor (SBTI), ethylenediaminetetraacetic acid (EDTA), pepstatin , CNBr-activated Sepharose 4B benzamidine, and trasylol were purchased from Sigma Chemical Company, St. Louis, MO. Immobilized *p*-aminobenzamidine support was purchased from Pierce Chemical Co., Rockford, IL.

Isolation of Trypsin and a Trypsin-Like Enzyme. "Trypsin fraction" containing both trypsin and trypsin-like enzymes was prepared from cunner pancreas by a combination of salt fractionation, acetone precipitation, and affinity chromatography on Sepharose 4B column as described previously (Simpson and Haard, 1985). Trypsin and trypsin-like enzymes in "trypsin fraction" were separated by a second affinity chromatographic step on an immobilized *p*-aminobenzamidine column described in

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0021-8561/87/1435-0652$01.50/0

detail elsewhere (Simpson and Haard, 1985).

Activity Measurements. The spectrophotometric procedure of Erlanger et al. (1961) was employed to determine the amidase activity of the trypsin or trypsin-like enzyme, using BAPA as substrate. The esterase activities of the two enzymes were also separately determined by the spectrophotometric procedure of Hummel (1959) with TAME as substrate. The molar concentrations of trypsin and trypsin-like enzymes were calculated from their molecular weights of 24K and 14K, respectively (Simpson and Haard, 1985).

pH Optima and Stability. The pH optima for BAPA hydrolysis were determined with the following buffer solutions: 0.1 M citrate–HCl, pH 2.0; 0.1 M citrate–NaOH, pH 4.0; 0.1 M citrate–NaOH, pH 6.0; 0.1 M Tris-HCl, pH 7.0; 0.1 M Tris-HCl, pH 7.5; 0.1 M Tris-HCl, pH 8.0; 0.1 M Tris-HCl, pH 8.5; 0.1 M Tris-HCl, pH 9.0; 0.1 M glycine–NaOH, pH 10.0; 0.1 M glycin–NaOH, pH 11.0. The influence of pH on the stability of the trypsin or trypsin-like enzyme was determined by preincubating the enzyme in various buffer solutions listed above in an ice bath for 30 min prior to assaying for residual activity.

Temperature Optima and Thermostability. The temperature optima and thermal stability for the amidase reaction was carried out as described elsewhere (Simpson and Haard, 1985). The Arrhenius energy of activation was calculated from activity data obtained at 5 °C intervals from 20 to 35 °C.

Influence of Inhibitors. The influence of the enzyme inhibitors PMSF, PCMB, EDTA, SBTI, trasylol, pepstatin, and benzamidine on the activity of cunner trypsin or the trypsin-like enzyme were separately determined by incubating each inhibitor with the enzyme at about 25 °C in 2 mM HCl for 30 min before application of BAPA or TAME and measurement of the initial rate of enzyme activity.

Amino Acid Composition of Cunner Enzymes. The enzymes were individually hydrolyzed in vacuo in 6 N HCl at 110 °C for 24, 48, and 72 h, and the resulting amino acids were separated on a Beckman 121 MB amino acid analyzer. Cysteine and methionine were determined separately by the method of Blackburn (1968), and tryptophan was determined by the method of Penke et al. (1974). The amino acid data were used to compute the acid/base ratios and the hydrophobicity indices (Bigelow, 1967) of the two proteases.

Kinetic Properties. Measurements of the initial rates of hydrolysis of various concentrations of BAPA (pH 8.2) were made at 20, 25, and 30 °C as described earlier by Simpson and Haard (1984). The range of substrate concentration used was 1.00–3.33 mM. Values of V_{max} and K_m' were estimated by the least-squares method of Hanes (1932). Physiological efficiency was calculated as described by Pollock (1965).

Hydrolysis of Native Protein Substrates. Approximately 200 mg of hemoglobin or ribonuclease A was introduced in a 5-mL reaction vessel of a pH-stat autotitration system (Metrohm Herisau Dosimat 655 and Impulsomat 614, Brinkman Instruments, Rexdale, Ontario). The protein was dissolved in water and the resultant mixture adjusted with 1 M NaOH and water to a final volume or 5 mL and pH 8.0. In all studies, a few drops toluene were added to the suspension and equilibrated with a thermostatically controlled water bath maintained at 30 °C prior to the addition of the enzyme(s). With "denatured" protein substrate studies, the following modifications were applied to the procedure: (i) heat-treated RNase was prepared by heating RNase solution to 60 °C and holding it at this temperature for 30 min prior to cooling to 30 °C and addition of enzyme; (ii) urea-treated hemoglobin was prepared by adding hemoglobin to 4 mL of 2 M urea (pH 8.0) and adjusting to pH 8.0 with 1.0 M NaOH. The enzymes used in the study were preadjusted to a final concentration of approximately 1 μg/mL, 400 L of the various enzyme stock solutions was added separately to the sample in the reaction vessel, and the reaction was followed by titrating with standard 0.05 N to a set pH of 8.0 at 30 °C. The reaction was continuously flushed with N_2 and left to proceed for a least 24 h to measure the degree of hydrolysis (DH), based on the procedure described by Novo Industries (Anonymous, 1978).

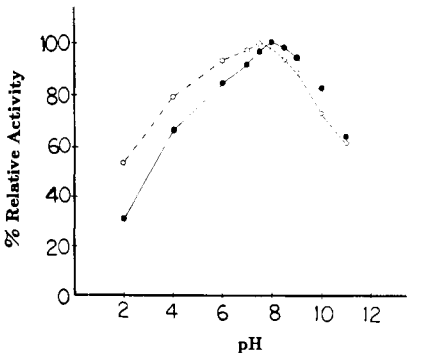

Figure 1. pH optima of cunner enzymes. Average values for triplicate determination: trypsin (●); trypsin-like (O--O). Trypsin concentration was 2 μg/mL and trypsin-like was 1.2 μg/mL in the assay media.

Inactivation of RNase by Proteases. The influence of the trypsin or trypsin-like enzyme on the activity of RNase was investigated as earlier described by Bang-Jensen et al. (1964). The enzymes were preadjusted to have similar activity on BAPA at 25 °C.

RESULTS AND DISCUSSION

pH Optima and Stability. The pH optima for the hydrolysis of BAPA at 25 °C were 8.0 for cunner trypsin and 7.5 for the trypsin-like enzyme (Figure 1). These values were similar to those previously reported for invertebrate trypsins (Jany, 1976; Yoshinada et al., 1985; Simpson and Haard, 1984a; Hjelmeand and Raa, 1982; Murakami and Noda, 1981; Chen et al., 1978; Gates and Travis, 1969), as well as for higher vertebrate trypsins (Erlanger et al., 1961; Simpson and Haard, 1984a; Vithayathill et al., 1961).

Regarding the influence of pH on the stability of the enzymes, cunner trypsin was most stable at neutral to slightly alkaline pH (pH range 6.5–8.5) and least stable at acid pH (4.0), similar to observations made with fish and other lower vertebrate or invertebrate trypsins (Jany, 1976; Yoshinada et al., 1985; Simpson and Haard, 1984a; Hjelmeland and Raa, 1982; Ooshiro, 1967). The trypsin retained more than 90% of its activity at pH 8.0 at 0 °C after 160 h. However, the trypsin-like enzyme was more stable at acid pH (range 2.0–4.0) and least stable at alkaline pH (range 7.0–10.0). With respect to stability at various pH values, the trypsin-like enzyme from cunner is similar to trypsin from mammals (Erlanger et al., 1961; Simpson and Haard, 1984a; Vithayathill et al., 1961).

Temperature Optima and Thermostability. The temperature optimum for the hydrolysis of BAPA was 45 °C for both the cunner trypsin and the trypsin-like enzyme. This temperature optimum is similar to those reported for trypsins from certain fish (Simpson and Haard, 1984a; Hjelmeland and Raa, 1982; Ooshiro, 1971), but lower than those reported for trypsins from mammals

Table I. Inhibition of Cunner Enzymes by Protease Inhibitors

inhibitor	substrate	molar ratio[a]	% inhibition trypsin	% inhibition trypsin-like
PMSF	TAME	2.50:1	22.54	16.04
		1.25:1	59.47	59.91
EDTA	TAME	2.00:1	00.95	00.00
		1.00:1	01.14	00.45
SBTI	BAPA	2.50:1	42.23	32.96
		1.25:1	70.64	61.92
pepstatin	BAPA	2.00:1	00.19	00.22
		1.00:1	00.57	00.45
PCMB	BAPA	2.00:1	00.95	01.34
		1.00:1	00.75	01.11
benzamidine	BAPA	2.00:1	46.02	00.89
		1.00:1	85.61	01.11
trasylol	TAME	2.00:1	35.42	40.31
		1.00:1	89.02	83.74

[a] Enzyme to inhibitor ratio. The enzymes were assayed for esterase or amidase activity as described in Materials and Methods. The data presented are average values for triplicate determinations.

(Erlanger et al., 1961; Simpson and Haard, 1984a; Vithayathill et al., 1961). Arrhenius plots gave straight lines below 45 °C for both enzymes; the values of r^2 of linear regression analysis were 0.97 for both cunner trypsin and cunner trypsin-like enzyme. E_a for hydrolysis of BAPA by the trypsin-like enzyme was 2054 J/mol and for trypsin was 1791 J/mol. These E_a values are similar to those reported for trypsin from Greenland cod (Simpson and Haard, 1984a) and mackerel (Ooshiro, 1971) but much lower than E_a values for trypsins from mammals, e.g. 3153 J/mol for bovine trypsin (Simpson and Haard, 1984b). Both cunner enzymes, especially trypsin, are very heat stable compared to trypsins from other fish (Simpson and Haard, 1984a,b; Hjelmeland and Raa, 1982; Ooshiro, 1971). Approximately 90% of the initial activity of the trypsin and 70% of that of trypsin-like enzyme were retained after 30-min incubation at 80 °C, and 60% and 40% activities were retained respectively after 30 min at 100 °C. The unusual heat stability of these enzyme is surprising since trypsins from fish thus far characterized tend to be readily inactivated by temperatures as low as 40–50 °C (Simpson and Haard, 1984a; Hjelmeland and Raa, 1982; Murakami and Noda, 1981; Ooshiro, 1971).

Influence of Inhibitors. Both enzymes were appreciably inhibited by PMSF, SBTI, and trasylol (Table I). Benzamidine inhibited cunner trypsin but did not appreciably influence the activity of the trypsin-like enzyme. The other inhibitors tested had negligible or not effect on either of the two enzymes. The inhibition of both enzymes by PMSF suggests that they are serine proteases like other trypsins and trypsin-like enzymes (Jany, 1976; Hjelmeland and Raa, 1982; Farhney and Gold, 1983; Bundy and Gustafson, 1973). The inhibition of the two enzymes by trasylol and SBTI suggests that these enzymes have a similar mechanism of substrate bonding by their active centers to other trypsins (Gates and Travis, 1969; Blow et al., 1974; Stambaugh and Buckley, 1972). However, the finding that benzamidine inhibits only the cunner trypsin but not the trypsin-like enzyme suggests that their active centers are probably not entirely identical. The cunner trypsin but not the trypsin-like enzyme probably has an active center homologous to that of authentic trypsins since the active centers of authentic trypsins are known to bind with guanidines and amidines with a resulting inhibition of their activities (Mihalyi, 1978; Mares-Guia and Shaw, 1965).

Amino Acid Composition. The amino acid composition of the cunner enzymes is summarized in Table II. The amino acid composition of cunner trypsin is similar to those of other trypsins from sources such as bovine, porcine, human, ovine, or shrimp (Kiel, 1971) or Greenland cod (Simpson and Haard, 1984a) with respect to (i) its high content of glycine, serine, glutamate, and aspartate and (ii) its relatively low content of methionine and basic amino acid residues. The trypsin-like enzyme is also rich in glycine, aspartate, serine, and threonine. The higher acid/base ratio of the cunner trypsin compared to the trypsin-like enzyme reflects the higher mole percentage of basic amino acid residues, lysine and arginine, and suggests that if all other things were equal, the 14000-Da protease should be more susceptible to autodigestion at the optimum pH of the enzyme because of the relative abundance of residues whose carboxyl linkages are susceptible to cleavage by trypsins and trypsin-like enzymes. This may explain why (i) the catalytic activity of cunner trypsin is more stable at neutral to alkaline pH than acidic pH like Greenland cod and shrimp trypsins, which also have relatively higher acid/base ratios, and (ii) the tryp-

Table II. Amino Acid Composition of Cunner Enzymes[a]

amino acid	trypsin 24 h	trypsin 48 h	trypsin 72 h	trypsin CV	trypsin IV	trypsin-like 24 h	trypsin-like 48 h	trypsin-like 72 h	trypsin-like CV	trypsin-like IV
Ala	15.73	14.80	15.34	15.29	15	13.12	13.48	13.43	13.34	13
Arg	1.74	2.12	2.22	2.03	2	2.03	2.15	1.78	1.99	2
Asp	24.78	22.73	23.38	23.63	24	13.11	13.50	13.35	13.32	13
Cys	11.07			11.07[a]	11	4.91			4.91[a]	5
Glu	16.18	15.43	15.83	15.81	16	9.21	9.45	9.46	9.37	9
Gly	27.68	25.67	26.60	26.65	27	14.26	14.41	14.54	14.40	14
His	2.98	2.88	3.09	2.98	3	1.32	1.28	1.35	1.32	1
Ile	11.94	14.31	14.94	13.73	14	5.24	5.52	5.43	5.40	5
Leu	14.49	14.37	14.76	14.54	15	11.44	11.61	11.64	11.56	12
Lys	13.29	16.37	13.32	14.33	14	8.18	8.42	8.34	8.31	8
Met	2.16			2.16[b]	2	1.15			1.15[b]	1
Phe	3.06	3.05	3.17	3.09	3	3.62	3.55	3.70	3.62	4
Pro	9.67	8.95	9.35	9.32	9	6.01	5.91	5.95	5.96	6
Ser	33.88	28.90	27.80	36.27[c]	36	11.51	10.09	9.13	10.24	10
Thr	10.14	10.18	9.77	10.03	10	12.34	12.81	12.70	12.62	13
Try	3.74			3.74[d]	4	3.17			3.17[d]	3
Tyr	11.38	8.95	9.95	10.00	10	2.63	2.68	2.70	2.63	3
Val	10.98	16.01	17.49	14.83	15	11.91	12.94	12.07	12.31	12
total					230					134

[a,b] Determined after performic acid oxidation (Blackburn, 1968). [c] Extrapolated to zero time after hydrolysis. [d] Determined by the method of Penke et al. (1977). [e] Key: CV = calculated value; IV = integral value.

Table III. Summary of Kinetic Properties of Cunner Enzymes

enzyme	assay temp, °C	K_m', mM	V_{max}[a]	V_{max}/K_m'[b]
trypsin	30	1.35 ± 0.06	310.4 ± 12.9	226
	25	0.73 ± 0.03	181.3 ± 7.9	251
	20	1.59 ± 0.06	131.4 ± 7.7	82
trypsin-like	30	2.34 ± 0.05	233.3 ± 5.9	99
	25	1.38 ± 0.06	164.1 ± 6.4	119
	20	1.93 ± 0.04	112.4 ± 2.8	58

[a] Turnover number is BAPA units/micromole of enzyme. Data presented are average values for triplicate determinations. One BAPA unit is defined as an absorbancy change of 2.933/min at 25 °C in a 3-mL reaction mixture. r^2 for regression of Lineweaver–Burk plots of data from 30, 25, and 20 °C assay temperatures were 0.97, 0.91, and 0.88 for trypsin and 0.94, 0.99, and 0.94 for the trypsin-like enzyme. [b] Physiological efficiency is the ratio V_{max} to K_m' at any given temperature (Pollock, 1965).

sin-like enzyme is more like bovine, ovine, or human trypsin in being more stable at acid pH than alkaline pH, as they all have relatively lower acid/base ratios.

However, the 14 000-Da protein had a relatively higher content of hydrophobic amino acid residues, in proportion to its size such that the calculated average hydrophobicity ($H\Phi_{av}$) of the trypsin-like enzyme (0.97 kcal/residue) was higher than that of the cunner trypsin (0.89 kcal/residue). Earlier we reported the $H\Phi_{av}$ for Greenland cod trypsin was 0.86 kcal/residue (Simpson and Haard, 1984a). On the basis of Bigelow's (1967) hypothesis relating $H\Phi_{av}$ to thermal stability, it would be predicted that cunner trypsin would have comparable thermal instability to Greenland cod trypsin, and this was not demonstrated experimentally as noted earlier on. However, the secondary and tertiary folding of cunner trypsin may differ considerably from other trypsins; e.g., fewer of its hydrophobic residues may be "buried" in the native state compared to what occurs in trypsins from other organisms.

Kinetic Properties of Cunner Enzymes. The kinetic parameters estimated from studying the influence of BAPA concentration on reaction rate and by the least-squares method (Hanes, 1932) are summarized in Table III. The substrate turnover numbers (V_{max}) were higher for the trypsin–BAPA reaction than the trypsin-like enzymes–BAPA reaction at all three temperatures investigated. The K_m' values for both enzymes varied considerably with assay temperature and in this respect were similar to Greenland cod trypsin (Simpson and Haard, 1984b). The K_m' values were higher fo the trypsin-like enzyme–BAPA reaction than the trypsin–BAPA reaction at all three easy temperatures. Furthermore, both enzymes exhibited K_m' values at 25 °C such that their physiological efficiencies were highest at this temperature and lower at 20 or 30 °C. The cunner enzyme differed from Greenland cod and bovine trypsins whose physiological efficiencies for BAPA hydrolysis increased from 25 to 35 °C (Simpson and Haard, 1984b).

Hydrolysis of Protein Substrates. The initial rates of hydrolysis of native hemoglobin and native ribonuclease A (Table IV) indicate that cunner trypsin hydrolyzed native protein substrates much more readily than the trypsin-like enzyme or bovine trypsin. However, after prolonged reaction, the trypsin-like enzyme hydrolyzed native proteins much more extensively (% DH) than either the cunner or bovine trypsin, suggesting that it probably has a broader specificity than the trypsins. Even though all three enzymes hydrolyzed denatured protein substrates more rapidly and more extensively than they did the corresponding native substrates, the catalytic rate of bovine trypsin on native protein substrates was considerably less

Table IV. Hydrolysis of Protein Substrates by Cunner Enzymes

enzyme	substrate	initial rate of hydrolysis[a]	DH, %
bovine trypsin	hemoglobin	0.73	0.96
cunner trypsin		1.24	0.95
trypsin-like		0.43	2.53
bovine trypsin	UT-hemoglobin[b]	4.02	2.83
cunner trypsin		2.14	1.68
trypsin-like		1.05	5.50
bovine trypsin	ribonuclease	0.25	0.60
cunner trypsin		0.67	1.22
trypsin-like		0.46	2.50
bovine trypsin	HT-ribonuclease[c]	1.27	1.41
cunner trypsin		1.69	1.29
trypsin-like		0.85	3.72

[a] In milliequivalents/minute per micromole enzyme. [b] Urea-treated hemoglobin. [c] Heat-treated ribonuclease. Data presented are average values for triplicate determinations.

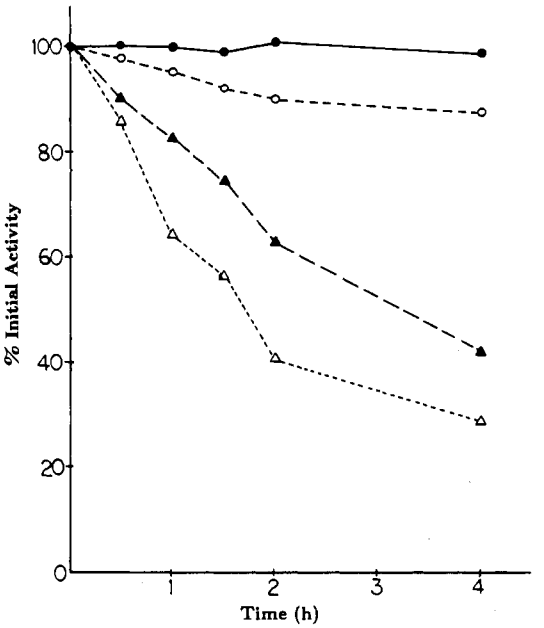

Figure 2. Inactivation of RNase by proteases. Average values of triplicate determinations: bovine trypsin (○); cunner trypsin (▲); trypsin-like (△); control (●). The enzymes, mixed with RNase, had equivalent activity with BAPA, i.e. 85 g, 4.6 g, and 10 μg/mL of reaction mixture respectively.

than that of cunner trypsin. For instance, bovine trypsin hydrolyzed native hemoglobin or RNase only 18% or 20% as fast as it did their denatured counterparts, while the rate at which cunner trypsin hydrolyzed the same native protein substrates was 58% or 40% compared to its hydrolysis of the denatured forms of the protein substrates. The trypsin-like enzyme hydrolyzed these native protein substrates 41% or 54% as fast as it did their denatured counterparts. A similar observation was made with regard to the DH study where the cunner enzymes hydrolyzed the native protein substrates more extensively than the bovine trypsin. This is evidenced by the fact that the DH of native hemoglobin or RNase substrate by bovine trypsin was 55% or 94% as compared to its DH of the denatured forms; the DH for the hydrolysis of native hemoglobin or RNase by trypsin-like enzyme was 46% or 67% as compared to its DH of the denatured forms.

Inactivation of RNase by Proteases. The rapid and more extensive hydrolysis of RNase was accomapanied by a loss of enzymic catalysis, similar to findings made with a trypsin-like enzyme from the stomachless crayfish

(Pfleiderer et al., 1967). Figure 2 indicates that the cunner enzymes acted to destroy the activity of RNase more so than bovine trypsin. Approximately 12% of RNase activity was lost with bovine trypsin while cunner trypsin destroyed about 58% and trypsin-like enzyme about 72% of RNase activity after 4-h incubation at 23 °C.

CONCLUSIONS

The trypsin and trypsin-like enzymes from stomachless cunner appear to be better suited for digestion of native proteins than is bovine trypsin. This conclusion is supported by the higher initial rates and/or degree of hydrolysis of native protein substrates by the cunner enzymes.

ACKNOWLEDGMENT

The assistance of P. Mansfield of the Biochemistry Department and R. Wilson of the Marine Sciences Research Laboraties at Memorial University, in procuring the samples used in the study, is gratefully acknowledged, as well as the excellent technical assistance of D. Hall and S. Banfield in carrying out the amino acid analysis work.

Registry No. BAPA, 6208-93-1; trypsin, 9002-07-7; *Tautogolabrus adspersus* serine proteinase, 109390-14-9.

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Received for review September 12, 1986. Accepted April 30, 1987.

Effect of Preliminary Thermal Treatment on the Digestion by Trypsin of Lupin Seed Protein

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The degradation by trypsin of lupin seed proteins (conglutin γ, globulin 6, globulin 8) of BSA and of casein is increased by preliminary heating of the protein (30 min at 100 °C). The effect differs with the protein considered. SDS–PAGE, TCA-soluble peptides, and amino acids liberated indicate rapid and massive breakdown of globulin 8 (a legumin) and of casein; degradation is less prevalent with BSA and develops even more slowly with globulin 6 (a vicilin). Least effect is with conglutin γ. Results are interpreted in terms of modifications of the protein structure.

The nutritional quality of a protein depends on bioavailability of essential amino acids; this in turn is affected to a large extent by the action of digestive enzymes. The efficiency of the latter is influenced by the treatments the protein undergoes, the most common of which is heating. Information on these items is still lacking for new food proteins as those of lupin seed.

Legume seed proteins are less susceptible to tryptic digestion than animal proteins (Kakade, 1974; Lynch et al., 1977a,b; Restani et al., 1983; Romero and Ryan, 1978). Several factors are involved, beside the presence of trypsin inhibitors. The primary structure, protein conformation, and presence of bound oligosaccharide all may contribute in decreasing the efficiency of proteolysis (Boonvisut and Whitaker, 1976; Fukushima, 1968; Kakade, 1974; Kakade et al., 1969; Lynch et al., 1977b; Romero and Ryan, 1978;

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0021-8561/87/1435-0656$01.50/0