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Comparison of Proteolytic Activities of the Enzyme Complex from Mammalian Pancreas and Pancreatin

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Abstract—The proteolytic activity and thermal stability of the enzyme complex of a cell suspension from pig and bovine pancreas glands was compared with those of pancreatin. The enzyme complex displayed the highest thermal stability and activity at 50°C. The kinetic constants, energies of activation and inactivation of the enzyme complex, and pH optimum (7.0 ± 0.1) at which this complex had the maximum proteolytic activity were determined. Pancreatin had a pH optimum of 8.0 ± 0.1.

The pancreas is known to contain enzymes with various substrate specificities, including trypsin (EC 3.4.21.4), α-chymotrypsin (EC 3.4.21.1), carboxypeptidase (EC 3.4.16.1), lipase (EC 3.1.1.3), and amylase (3.2.1.13) isolated in the form of an enzyme complex (pancreatin). Pancreatin is widely used in the medical and food industries due to its wide substrate specificity and the ability to hydrolyze proteins, fats, and polysaccharides of various chemical compositions [1].

The temperature dependence and some kinetic parameters of the effects of pancreatin were determined [2, 3]. However, isolation and purification of pancreatin include chemical and technological steps that increase the cost of this preparation. Instead of individual enzymes and complex enzyme preparations, animal or microbial cells have been used for transformation of steroids, obtaining amino acids, and designing enzyme electrodes over the last 20 years [4, 5]. Enzyme systems of living cells have some advantages, including the presence of cofactors (thiamin, riboflavin, nucleotides, etc.) and their high resistance to changes in the medium pH.

However, quantitative characteristics of the enzyme complex from mammalian pancreas under various conditions have received little attention [6, 7]. Therefore, studies of the proteolytic activity and thermal stability of cell suspensions from mammalian pancreas (PCS) in buffer solutions are of considerable interest.

This work was designed to estimate the activity of enzyme complexes of pig and bovine PCS and to determine the conditions of their use for protein hydrolysis.

MATERIALS AND METHODS

Pig and bovine pancreases (Ramenskii Meat Packing Plant, Russia) and medicinal pancreatin with a proteinase activity of 5000 U per g enzyme preparation and a protein content of 90% were used. Table 1 shows the amino acid composition of dried protein extract (DPE) containing 86% protein and 2.5% fat, which was obtained from meat–bone forcemeat [8] and used as a substrate for hydrolysis.

Suspensions with a dilution coefficient (water module) of 0.25–7.50 were prepared from pancreas samples of various animals. Thawed and minced pancreases (100 g) were homogenized with 25, 50, 100, 150, 250, 500, and 750 ml of distilled water. Ethanol (3 vol %) was added to the suspension as a preservative. The proteolytic activity of PCS was determined by a modified method of Anson [9] using 1%-aqueous water solution of sodium caseinate as a substrate.

Table 1. Amino acid composition of dried protein extract from meat–bone forcemeat, g/100 g protein [7]

Amino acid	Extract of meat–bone forcemeat	Egg white (standard)
Aspartic acid	7.02	7.0
Threonine	2.12	5.0
Serine	2.79	8.4
Glutamic acid	13.31	12.4
Proline	7.57	4.2
Glycine	10.53	3.5
Alanine	6.96	6.7
Cystine	0.04	2.3
Valine	4.13	7.9
Methionine	1.76	3.1
Isoleucine	2.18	6.6
Leucine	5.00	8.8
Tyrosine	4.56	4.3
Phenylalanine	5.12	5.8
Histidine	8.13	2.4
Lysine	2.96	6.4
Arginine	8.27	2.4
Total content	92.45	98.9

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Table 2. Proteolytic activity of enzyme complexes of pig and bovine PCS

Water module	Proteolytic activity, U/g × 10^3	
	pig PCS	bovine PCS
7.50	1.03 ± 0.07	0.85 ± 0.04
5.00	1.26 ± 0.08	1.08 ± 0.03
2.50	2.23 ± 0.05	1.91 ± 0.04
1.50	3.63 ± 0.14	2.98 ± 0.05
1.00	5.20 ± 0.40	4.24 ± 0.12
0.50	7.90 ± 0.35	5.01 ± 0.15
0.25	7.03 ± 0.57	4.48 ± 0.34

The constants of activation and inactivation of PCS enzyme complex were estimated by incubating 1% pancreas forcemeat suspensions in 0.1 M Tris buffer at 45–55°C and pH 7.0 for 6 h. Samples (1 ml) for measuring the proteolytic activity were taken at 30-min intervals.

Hydrolysis of 3% DPE was performed in a thermostatic flask at the enzyme/substrate protein ratio of 1 : 20, 50°C, pH 7.0, with constant mixing for 4 h.

The pancreas was minced and homogenized in water (weight ratio 2 : 1) to prepare PCS for hydrolysis. Enzyme complexes of bovine and pig PCS were activated by preincubation at 45°C for 1.5 h and at 50°C for 2 h, respectively.

The amino acid composition of hydrolysates was estimated in an LC-3000 amino acid analyzer (Eppendorf-Biotronic, Germany).

RESULTS AND DISCUSSION

Table 2 shows the dependence of proteolytic activities of pig and bovine PCS on the water module.

The maximum proteolytic activity was observed at a low dilution of the pancreas forcemeat with water (water module 0.50). The total enzyme activity of pig PCS was 15–50% higher than that of bovine PCS. Therefore, pig PCS were used for obtaining protein hydrolysates.

Enzyme activities of pig and bovine PCS remained nearly unchanged at room temperature for two to three days, whereas the activity of pancreatin decreased markedly under these conditions [3]. At temperatures above 65°C, pancreatin and PCS enzyme complex did not display proteolytic activity.

The proteolytic activity of pig PCS enzymes was maximum at 50°C and remained unchanged for 2 h (Fig. 1). An increase or decrease in temperature reduced the enzyme activity. The thermal stability of PCS enzyme complex decreased with an increase in temperature.

The temperature dependence of the enzyme activity of bovine PCS did not have a plateau phase (Fig. 2). Incubation of bovine PCS at 45–55°C led to an initial increase and further decrease in the enzyme activity. Table 3 shows the kinetic constants of stability.

Kinetic constants of activation and inactivation (Table 3) were calculated from logarithmic anamorphoses of temperature dependences of activation and inactivation of PCS enzyme complex (Figs. 1b, 2b). Energies of activation (E_a) and inactivation (E_a^{in}) of the proteolytic enzyme complex were determined from Arrhenius plots in $\ln K - 1/T$ coordinates (Fig. 3). Pancreatin, pig PCS, and bovine PCS had various inactivation constants. PCS proteolytic enzymes were more stable than pancreatin and were almost independent of the animal species.

Figure 4 shows the dependence of the enzyme complex proteolytic activity on the medium pH. Pancreatin had a pH optimum of 8.0 [3]. Enzyme complexes of pig

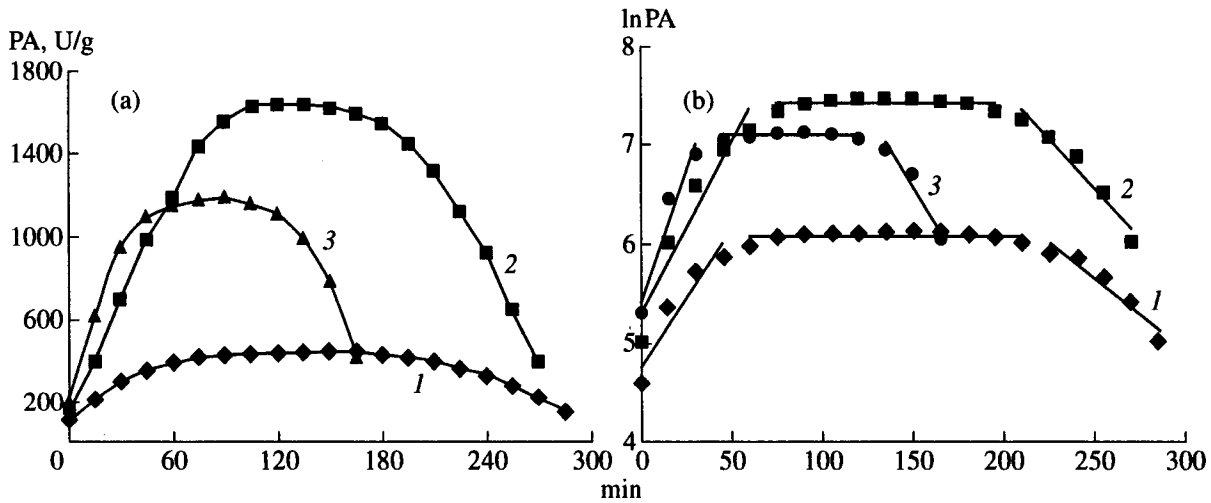

Fig. 1. Dependence of proteolytic activity (PA) of pig PCS enzymes on time of cultivation (a) and its anamorphoses in semilogarithmic coordinates (b) at temperatures of (*1*) 45, (*2*) 50, and (*3*) 55°C.

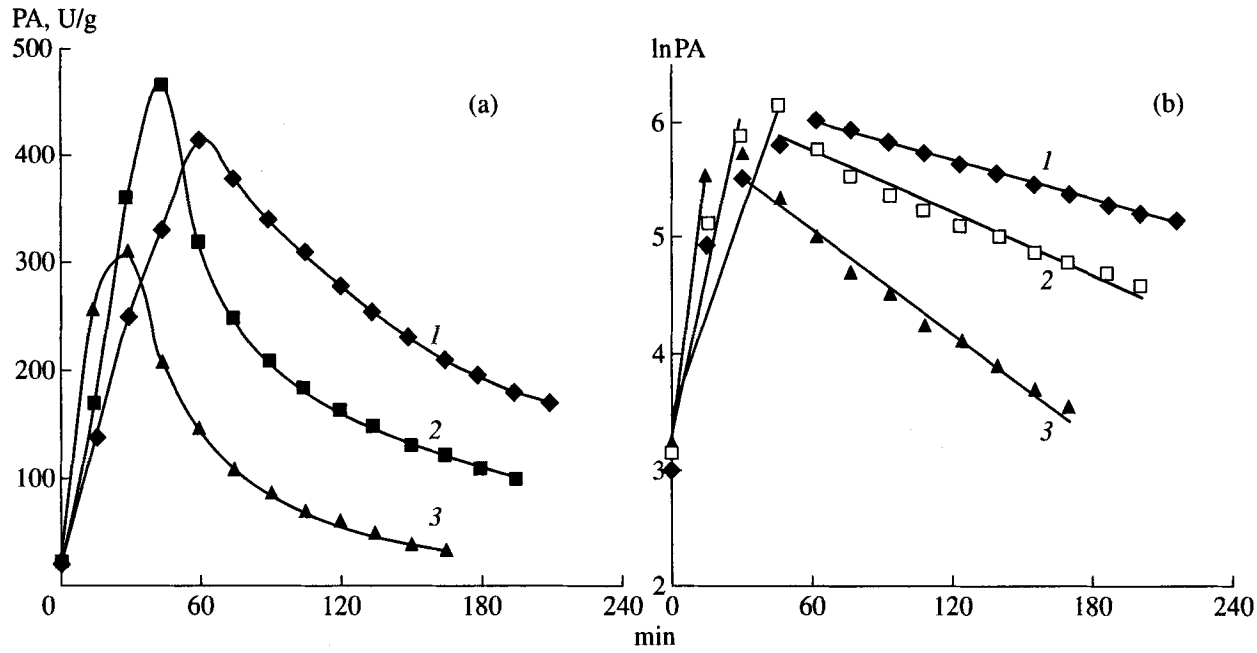

Fig. 2. Dependence of the proteolytic activity (PA) of bovine PCS enzymes on time of cultivation (a) and its anamorphoses in semi-logarithmic coordinates (b) at temperatures of (*1*) 45, (*2*) 50, and (*3*) 55°C.

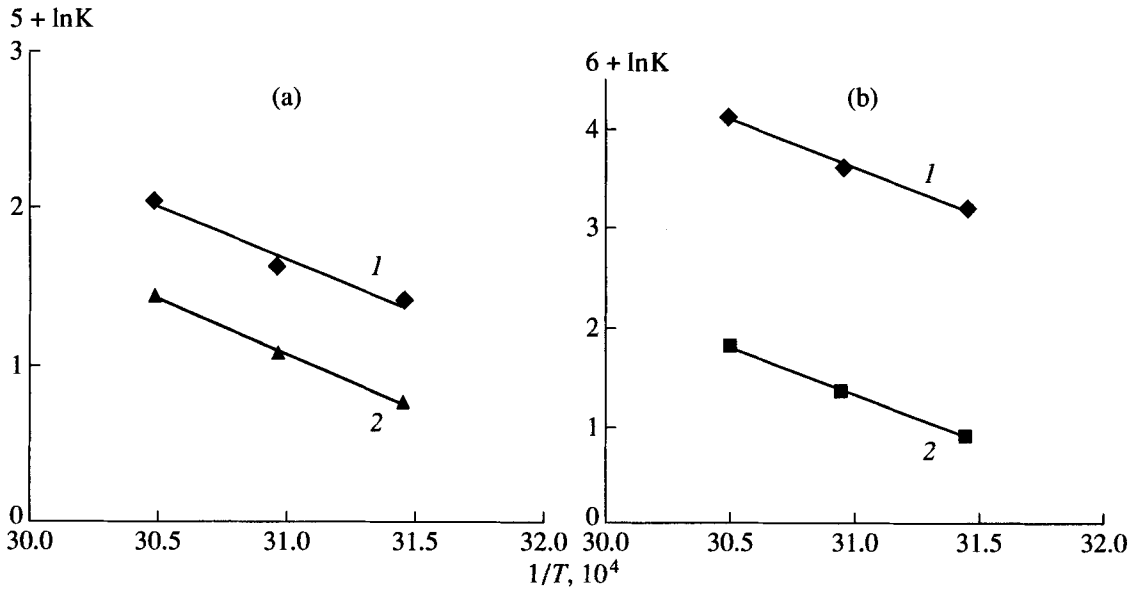

Fig. 3. Energies of (*1*) activation and (*2*) inactivation of pig (a) and bovine (b) PCS enzymes.

and bovine PCS had a pH optimum of 7.0, which probably indicated that the pancreatic enzymes were in the immobilized state.

These data showed that the method for activation of the enzyme complex of mammalian PCS consisting of their preliminary thermostatic treatment was elaborated (Materials and methods section). Activated enzyme preparations of bovine and pig PCS displayed proteolytic activities of 6000 and 9000 U/g, respectively.

Thus, the activity of PCS enzyme preparations surpassed that of dried pancreatin-containing PCS with a proteinase activity of 5000 U/g [3] by 1.2- to 1.8-fold.

Studies of pancreatin and PCS enzyme complex were performed using DPE of the meat–bone forcemeat (agricultural food additive). The hydrolysis of 3% DPE was performed using various enzyme preparations (pancreatin and nonactivated and activated enzyme complexes of pig PCS). Table 4 shows that the ratio of

Table 3. Constants of activation and inactivation energies of PCS enzyme complex and pancreatin

Preparation	Temperature, °C	Activation of PCS enzyme complex		Inactivation of PCS enzyme complex	
		constant of activation, $K_a \times 10^2$, min^{-1}	E_a, kJ/mol	constant of inactivation, $K_{in} \times 10^2$, min^{-1}	E_a^{in}, kJ/mol
Pig PCS	45	2.74 ± 0.08		1.44 ± 0.02	
	50	3.38 ± 0.11	55.15 ± 0.86	1.96 ± 0.03	60.24 ± 0.32
	55	5.19 ± 0.13		2.98 ± 0.04	
Bovine PCS	45	5.99 ± 0.56		0.61 ± 0.01	
	50	9.17 ± 0.55	81.39 ± 0.85	0.93 ± 0.01	81.21 ± 0.45
	55	15.35 ± 0.46		1.56 ± 0.03	
Dried pancreatin*	50	–**		9.30 ± 0.50	
	55	–	–	13.00 ± 0.70	84.80 ± 4.20
	60	–		26.70 ± 1.30	

* Rapid inactivation of native pancreatin [3].
** No data.

Table 4. Amino acid composition of DPE hydrolysates obtained by using pancreatin and nonactivated and activated pig PCS complexes

Amino acid	Content in hydrolysate, g/100 g protein			Amino acid	Content in hydrolysate, g/100 g protein		
	hydrolysis with pancreatin	hydrolysis with nonactivated enzyme complex of pig PCS	hydrolysis with activated enzyme complex of pig PCS		hydrolysis with pancreatin	hydrolysis with nonactivated enzyme complex of pig PCS	hydrolysis with activated enzyme complex of pig PCS
Aspartic acid	1.02	1.32	1.40	Isoleucine	1.00	1.33	1.52
Threonine	1.24	1.61	1.82	Leucine	3.49	4.49	4.93
Serine	1.32	1.75	1.97	Tyrosine	2.79	3.63	4.02
Glutamic acid	2.99	3.89	4.13	Phenylalanine	2.41	3.17	3.53
Proline	0.63	0.79	1.12	Histidine	2.69	3.49	3.83
Glycine	1.88	2.45	2.80	Lysine	2.32	2.99	3.28
Alanine	2.18	2.81	2.95	Arginine	5.79	7.49	8.12
Cystine	0.01	0.01	0.01	Tryptophan	0.49	0.58	0.63
Valine	2.61	3.47	3.92	Total content	36.02	46.78	51.63
Methionine	1.16	1.51	1.65				

amino acids was similar in all hydrolysates. The use of enzyme preparations of pig PCS resulted in a 45–50% yield of amino acids. This yield surpassed that observed after the use of dried pancreatin by 1.3- to 1.5-fold. It should be emphasized that preliminary activation of PCS enzyme complex enhanced the efficiency of hydrolysis.

Thus, our studies showed that activated enzyme complexes of PCS should be used for obtaining enzyme

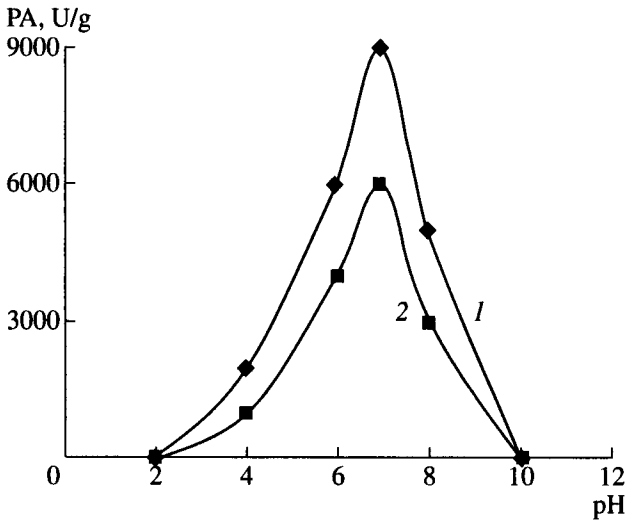

Fig. 4. Dependence of activities of enzyme complexes of pig (*1*) and bovine (*2*) PCS on medium pH.

hydrolysates from animal materials under mild conditions.

REFERENCES

1. Mashkovskii, M.D., *Lekarstvennye sredstva* (Drugs), Moscow: Meditsyna, 1993, vol. 2, p. 63.

2. Neklyudov, A.D., Ilyukhina, V.P., Mosina, G.I., Petrakova, A.N., Fedorova, N.V., and Kuznetsov, V.D., *Prikl. Biokhim. Mikrobiol.*, 1996, vol. 32, no. 2, pp. 231–236.

3. Neklyudov, A.D., Ivankin, A.N., and Baburina, M.I., *Prikl. Biokhim. Mikrobiol.*, 1998, vol. 34, no. 1, pp. 61–65.

4. Skryabin, G.K. and Koshcheenko, K.A., *Biotekhnologiya* (Biotechnology), Baeva, A.A., Ed., Moscow: Nauka, 1984, pp. 70–77.

5. *Immobilized Cells and Enzymes: a Practical Approach*, Woodward, J., Ed., Oxford: IRL, 1985. Translated under the title *Immobilizovannye kletki i fermenty*, Vudvorda, Dzh., Ed., Moscow: Mir, 1988.

6. Kalmykov, L.E. and Golubev, T.I., *Sov. Med.*, 1956, no. 3, pp. 66–69.

7. Krylov, I.A., Krasnoshtanova, A.A., and Manakov, M.N., *Biotekhnologiya*, 1998, no. 6, pp. 63–68.

8. Neklyudov, A.D., Ivankin, A.N., Berdutina, A.V., Karpo, B.S., Baer, N.A., and Dubina, V.I., *Myasn. Ind.*, 1998, no. 6, pp. 42–44.

9. Rukhlyadeva, A.P. and Polygalina, G.V., *Metody opredeleniya aktivnosti gidroliticheskikh fermentov* (Determination of Hydrolytic Enzyme Activity: Methods), Moscow: Legkaya i Pishchevaya Prom–st', 1981.