IMPROVEMENT OF THERMOSTABILITY OF PANCREATIN - ENZYME OF BEEF CATTLE PANCREAS

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SUMMARY

The method for pancreatin immobilization on carboxylmethylcellulose is described. Optimal conditions for reaction are defined: concentrations of reagents, temperature, pH, etc., all that allows to produce conjugate of an enzyme with a polymer, preserving up to 85% of protease and 92% of esterase activities.

It is shown that native and immobilized pancreatins have thermolabile and thermostable fractions with their inactivation constants. It is established that immobilization of pancreatin increases its thermostability by 1.5 - 3 times.

INTRODUCTION

At present protein catalysts-enzymes are widely used in food industry. One of these enzyme preparations is pancreatin, derived from beef cattle pancreas. To increase time of activity and thermostability of the enzyme it can be immobilized on one of the water-soluble polymer carriers.

The aim of the present work was to study conditions of pancreatin fixation on carboxylmethylcellulose (CMC), which is allowed for use in food industry, and also to investigate properties of the obtained conjugate.

MATERIALS AND METHODS

As objects of research served CMC polymeric matrix with molecular weight (mw) 65-70 kDa and pancreatin of "medical"grade having protease activity 5000 units/g of enzyme preparation and protein content 90%. Protein content was determined by Lowry's method (Lowry et al., 1951), total activity of proteolysis - by Anson's method (Vasilyeva et al., 1976), using casein by Hamersten as a substrate, or by hydrolysis of 2,4,6 - trinitricbenzolsulphoacid (Adler Nissen, 1979), esterase activity - by rate of hydrolysis of ethyl ester or benzolarginine on pH-state "Radiometer" (Denmark). Molecular weight of the obtained conjugate was determined by gel-filtration on a Sephadex column G-100. Thermostability of native and immobilized pancreatin was evaluated by changes of activity of solutions in 0.1 M tris-buffer at pH 7.5 and temperature 45-70°C. Solution of conjugate pancreatin-CMC was lyophilized, white powder was obtained as a result, showing characteristics given in Table 1.

TABLE 1 Characteristics of immobilized pancreatin

N of batch	Weight of conjugate	Specific activity, units		Total activity, units		Content of pancreatin,%	Yield by protein, %	Yield by activity,%	
		esterase	protease	esterase	protease			esterase	protease
1									
2	6.20	91008	80.7	564250	500	12.5	50	90	80
3	6.24	83638	74.5	521900	465	12.4	54	87	75
	6.25	96720	85.6	604500	535	12.6	59	92	85

TABLE 2 Thermodynamic constants of native and immobilized pancreatin [1]

Enzymic preparations	Thermodynamic constants	Temperature, °C					
		45	50	55	60	65	70
Native preparation	1. Constant of inactivation of fast stage $K_i \cdot 10^2$ min^{-1}	-	9.3±0.5	13.0±0.7	26.7±1.3	-	-
	2. Constant of inactivation of slow stage $K_i \cdot 10^2$ min^{-1}	1.3±0.07	0.73±0.04	0.8±0.04	0.73±0.04	-	-
	3. Activation energy of inactivation process, kJ/mole			84.8±4.2 (fast stage) 66.2±3.3 (slow stage)			
Immobilized preparation	1. Constant of inactivation of fast stage $K_i \cdot 10^2$ min^{-1}	-	-	2.6±0.1	5.3±0.3	14.0±0.7	22±1.1
	2. Constant of inactivation of slow stage $K_i \cdot 10^2$ min^{-1}	-	0.22±0.01	0.67±0.03	0.41±0.02	0.47±0.02	1.5±.08
	3. Activation energy of inactivation process, kJ/mole			113.1±5.7 (fast stage) 121.4±6.1 (slow stage)			

[1] Incubation of enzymes was conducted in 0.1 M tris-buffer, pH 7.5

RESULTS AND DISCUSSION

It is known that one of the most simple and reliable nethods of enzyme fixation on the carrier surface is formation of covalent link of functional carrier groups with free amino- and carboxyl groups of an enzyme.

For activation of carboxyl groups of CMC, water-soluble carbodiimids can be used, such as 1-ethyl-3- (3 - dimethylaminopropyl) - carbodiimid (CD), which upon accomplishment of the fixation process is removed from reaction medium by dialysis of the obtained conjugate against distilled water (Shreder and Lubke, 1967). In our case this method of fixation was chosen.

The highest soluble concentration of CMC in 0.05 M phosphate buffer at pH 8.2 is 2.5 x 10^{-2}M, of pancreatin - 1 x 10^{-4}M. The reaction of linking of enzyme with carrier at these concentrations during 5 hours at t=4°C showed, that pancreatin bounds with polymer by 25% of its initial amount, preserving enzymic activity to 95%.

When pH of the buffer solution is lowered to 7.5, CMC is solubilized to 5 x 10^{-4}M concentration, pancreatin - to 1 x 10^{-4}M, CD - to 2 x 10^{-2}M. Incubation at these concentrations during 5 hours at t=4°C increases the amount of enzyme, linked with carrier, to 45% of the initial value.

Increase of incubation time to 18 hours under above-mentioned conditions allows to increase the amount of linked protein to 54-59%, achieveing activity of 88-92% of the initial value. Further increase in incubation time of reaction mix to 48 hours didn't raise the amount of CMC-linked pancreatin, and enzymic activity lowered to 80% of the initial value. Thus, the optimum conditions of the reaction are: concentration of CMC - 5 x 10^{-4}M, of pancreatin - 1 x 10^{-4}M, of CD - 2 x 10^{-2}, incubation time - 18 hours at t=4°C.

The obtained conjugate of pancreatin with CMC contains 3 fractions: one - with mw 27-30 kDa,

corresponding to the main enzymes being present in pancreatin composition - that is to trypsin and chymotrypsin, which remained in conjugate not linked with CMC by amid links and two fractions with mw above 67-70 kDa, probably corresponding to the same enzymes and linked with CMC by amid links. The amount of CMC-linked enzymes equals 50%.

Optimum pH of the native pancreatin is in the range of 7.5-7.8; optimum pH of immobilized pancreatin shifts to the more acid side, being in the range of 7.0-7.3, which should be taken into account during further research of kinetic and other characteristics of immobilized enzyme.

Fig. 1 amd 2 show curves of dependencies of thermostability of model solutions of native and immobilized pancreatin in 0.1 M tris-buffer. It is interesting to note, that both native and immobilized pancreatin have two clearly expressed functions - thermolabile and thermostable ones. Similar dependence is characteristic of the most part of immobilized enzyme preparations, however, it is not often observed in case of native enzyme, which probably can be explained by self-immobilization of enzymes being part of pancreatin composition. This phenomenon is probably caused by hydrophobic protein-protein interrelation under Van der Wal forces effect. It can be supposed that prolonged activity of native pancreatin in a human organism is due to the presence of a thermostable fraction in it.

Inactivation of both native and immobilized pancreatin subjects to the Arrenius equation in the temperature range 45-65°C. The obtained inactivation constants and activation energy of the process are given in table 2. At the temperature below 50°C for native enzyme and

Fig. 1 Dependence of activity logarythm of native pancreatin on incubation time in 0.1 M tris-buffer

1 - 40°C, 2 - 45°C, 3 - 50°C, 4 - 55°C, 5 - 60°C

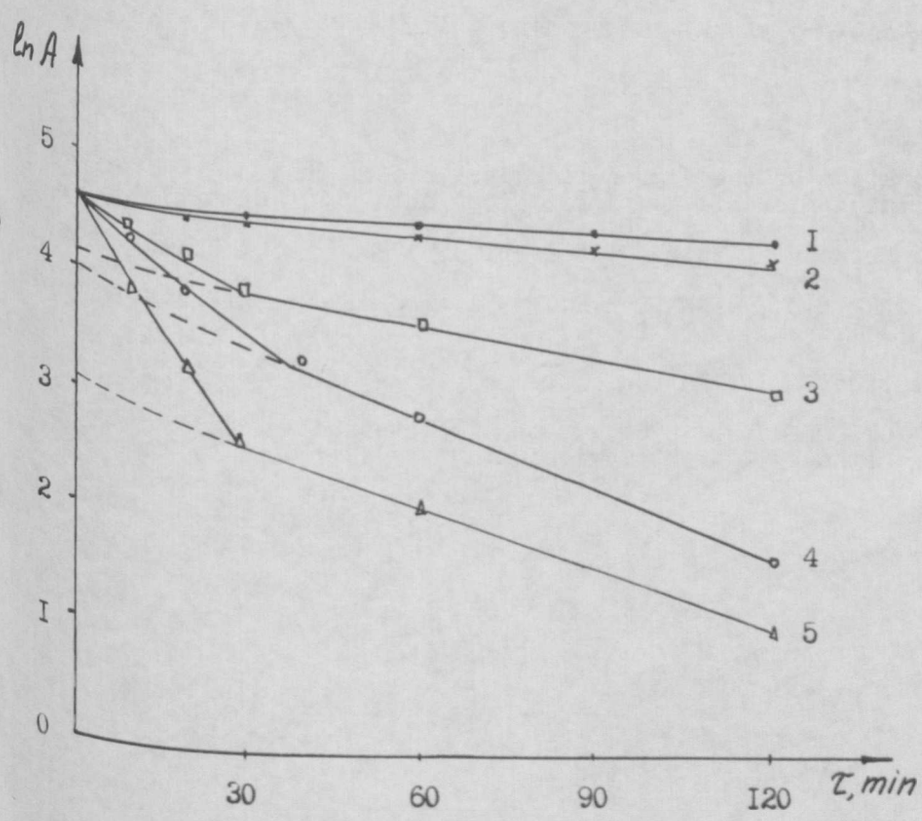
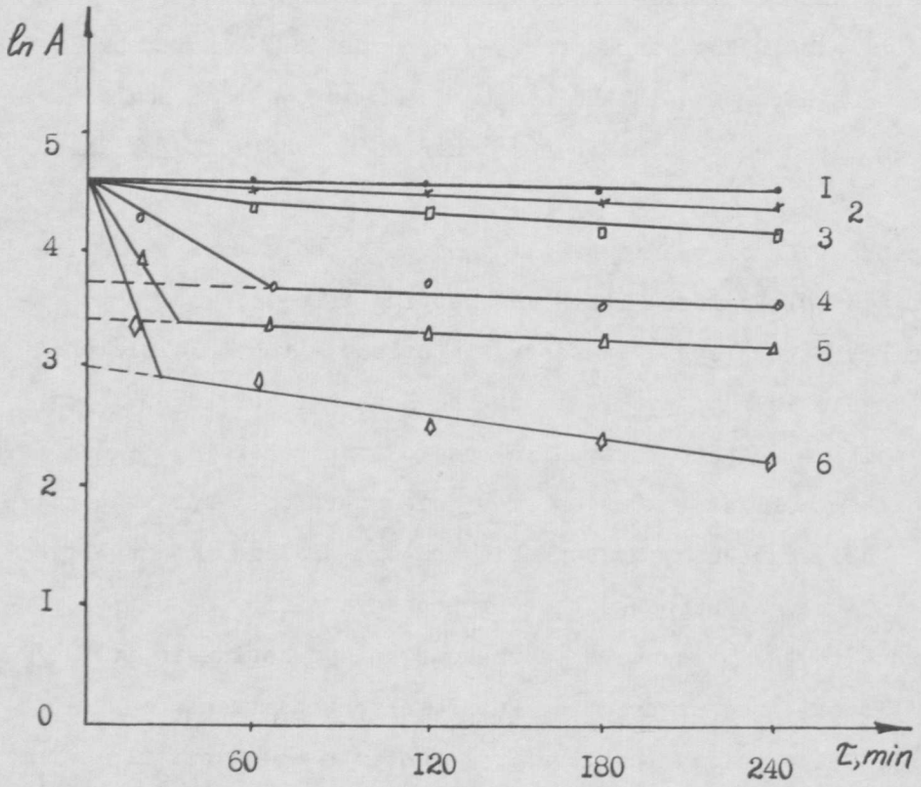


Fig. 2 Dependence of logarythm of activity of immobilized pancreatin on time of incubation in 0.1 M tris-buffer

1 - 45°C, 2 - 50°C, 3 - 55°C, 4 - 60°C, 5 - 65°C, 6 - 70°C



below 55°C for immobilized enzyme both fractions merge into some "mean" one with the corresponding mean inactivation constants. As seen from table 2, immobilization of pancreatin increases its thermostability by 1.5-3 times, and consequently, increases time of its action.

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