

The effect of temperature, acidification, and alkalization changes as well as ethanol on salivary cathepsin D activity

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Abstract

The activity of salivary cathepsin D undergoes inactivation at the temperature of 50-60°C and at pH of 2.0 and pH of 8.0-10.0. The enzyme activity is also decreased by high concentrations of ethanol and high-proof alcoholic beverages. The factors should be taken into consideration in the evaluation of salivary cathepsin D activity.

Key words: human saliva, cathepsin D, temperature, pH, ethanol.

Introduction

Cathepsin D occurs in human saliva [3]. The changes of its activity in the saliva can play an important role in the patho-biochemistry and diagnostics of salivary gland, gingiva, and oral mucosa diseases. External physical and chemical factors can affect the activity of this protease in the saliva.

The aim of the study was to determine the influence of high and low temperature, acidification, and alkalization as well as ethanol and alcoholic beverages on the activity of mixed saliva cathepsin D.

Material and methods

Ethanol, (POCh Gliwice); hemoglobin (Difco Laboratories, USA); alcoholic beverages: moonshine (home-made), Napoleon cognac (French product), EB beer (Elbląg brewery), Herbowe, Magnat, Porter, Złote beer (Dojlidy brewery – Białystok), Pałacowy brandy (Polmos, Białystok) apple, currant, strawberry wine (home-made), Premium, Zbożowa, Żubrówka, Żytnia vodka (Polmos, Białystok). Alcohol content in alcoholic beverages was determined using an alcoholometer kit [2]; Folina and Ciocalteau reagent (Merck, Germany); copper reagent, prepared according to [1]; other reagents – POCh, Gliwice.

The mixed saliva was collected in fasting state from 12 adults (6 women and 6 men), it was not centrifuged and stored in -75°C. Before the examination, particular saliva samples were mixed with the use of a flow homogenizer. The content of proteins in the saliva was mean 2.8 mg/ml and pH was 6.5.

1. The influence of temperature on salivary cathepsin D activity

The saliva (2 ml) was incubated at 10, 20, 30, 40, 50, 60, 70, and 80°C for 10 min. After incubation, the saliva was cooled with water and ice and pH was brought to 3.5. The amount of 0.1 ml of 6% hemoglobin was added to 0.4 ml of the sample and incubated for 6 hours at 37°C. The reaction was interrupted by adding 0.5 ml of 10% trichloroacetic acid. The samples precipitated at time 0 were considered to be the controls. The content of acid-soluble hemoglobin degradation products was determined in supernatant fluid obtained by centrifugation [4].

2. The influence of pH on salivary cathepsin D activity

The saliva (2 ml) was brought to 2.0-10.0 pH with divisions every 1.0 of pH unit with the use of 0.1 mol/l of HCl or NaOH and incubated at 37°C for 10 min. After incubation, the samples were brought to 3.5 of pH. The procedure was completed as in point 1.

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Table 1. The influence of 10 min incubation of the saliva at various values of temperature on cathepsin D activity

Temperature, °C	Cathepsin D activity	
	Tyr, nmol/l	%
0.0	248.0 ± 20.4	100.8
10.0	245.0 ± 23.6	99.6
20.0	249.0 ± 20.8	101.2
30.0	246.0 ± 20.6	100.0
40.0	216.2 ± 20.1	87.9
50.0	140.0 ± 15.2	56.9
60.0	8.0 ± 0.9	3.3
70.0	0.0	0.0
80.0	0.0	0.0

Table 3. The effect of various concentrations of ethanol on salivary cathepsin D activity

Ethanol % w/v	Cathepsin D activity	
	Tyr, nmol/l	%
0.0	186.4 ± 19.8	100.0
0.625	185.2 ± 18.6	99.4
1.25	180.4 ± 17.4	96.8
2.5	152.6 ± 15.0	81.9
5.0	120.2 ± 13.1	64.5
10.0	92.6 ± 9.8	49.7
20.0	86.0 ± 9.1	46.1

3. The effect of various ethanol concentrations on salivary cathepsin D activity

The amounts of 0.1 ml of ethanol of the concentrations from 3.12 to 100% w/v and 0.1 ml of 6% hemoglobin were added to 0.3 ml of the saliva and incubated at 37°C for 6 hours. All reagents were of 3.5 of pH. The procedure was completed as in point 1.

4. The influence of various alcoholic beverages on salivary cathepsin D activity

The amounts of 0.1 ml of an alcoholic beverage and 0.1 ml of 6% hemoglobin were added to 0.3 ml of the saliva and incubated at 37°C for 6 hours. All reagents were of 3.5 of pH. The procedure was completed as in point 1.

Results and discussion

It was observed that the temperature from 0 to 40°C did not affect the activity of salivary cathepsin D (Tab. 1). The temperature of 50-60°C significantly lowered the activity of this enzyme while the temperature from 70°C and higher inactivated it. Salivary cathepsin D activity was markedly decreased at 2.0 of pH (Tab. 2). The enzyme was stable at 3.0-7.0 of pH whereas at pH of 8.0 and higher – it underwent inactivation. Ethanol in the concentration of 5.0-20.0% w/v lowered the activity of salivary cathepsin D (Tab. 3), as did high-proof alcoholic beverages (Tab. 4). However, beer and wine did not have any effect on the activity of this enzyme.

Table 2. The influence of 10 min acidification and alkalization of salivary cathepsin D

pH	Cathepsin D activity	
	Tyr, nmol/l	%
2.0	9.8 ± 1.2	4.2
3.0	98.0 ± 10.4	42.1
4.0	233.0 ± 23.1	100.0
5.0	228.9 ± 25.4	98.2
6.0	224.6 ± 24.2	96.4
7.0	220.0 ± 21.1	94.4
8.0	140.4 ± 12.0	60.2
9.0	18.6 ± 2.0	8.0
10.0	0.0	0.0

Table 4. The effect of alcoholic beverages on salivary cathepsin D activity

Beverage	Ethanol concentration, % w/v	Cathepsin D activity	
		Tyr, nmol/ml	%
Control	0.0	186.2 ± 19.6	100.0
Moonshine	7.9	96.4 ± 10.2	51.8
Napoleon cognac	4.8	132.0 ± 11.8	70.9
EB beer	0.9	184.5 ± 19.2	99.1
Herbowe beer	1.1	188.0 ± 18.4	100.9
Magnat beer	1.2	179.8 ± 18.0	96.6
Porter beer	1.7	184.6 ± 19.2	99.1
Złote beer	1.1	182.6 ± 18.8	98.1
Pałacowy brandy	5.9	148.5 ± 15.0	79.7
Apple wine	2.5	158.4 ± 15.6	85.1
Currant wine	2.3	158.9 ± 15.2	85.3
Stawberry wine	1.8	167.4 ± 16.0	89.9
Premium vodka	7.8	96.0 ± 10.4	51.6
Zbożowa vodka	6.5	124.2 ± 13.8	66.7
Żubrówka vodka	5.2	132.0 ± 12.0	70.9
Żytnia vodka	6.7	126.0 ± 13.6	67.7

The decrease in the activity of cathepsin D at the temperature above 50°C and pH of 8.0-10.0 was also observed in cases of the enzyme derived from other sources [5,9]. The degree of salivary cathepsin D activity decrease by ethanol and alcoholic beverages depended on their concentrations [8]. The inhibition of gastric and pancreatic proteolytic enzymes was also observed [6,7,10].

The influence of physical and chemical factors on cathepsin D activity should be considered while determining the enzyme activity in the saliva for the evaluation of the salivary gland functioning as well as the diagnostics of the oral cavity diseases.

References

- Barret AJ. Proteinases in mammalian cells and tissues. North-Holland Publ Comp, Amsterdam, 1977; 240-3.
- Gacko M, Jurkowski J, Worowski K. The effect of chemical compounds found in strong drinks on hemostasis. Part I: Characteristics of the strong drinks. *Bromat Chem Toksykol*, 1995; 28: 39-43.
- Greczaniuk M. Cathepsin D of human saliva. XXXIV Congr Pol Biochem Soc, Białystok, 1998; 87.
- Greczaniuk A, Roszkowska-Jakimiec W, Gacko M, Worowska A. Determination of cathepsin D activity in blood plasma using hydrochloric acid denatured haemoglobin. *Diagn Lab*, 2000; 36: 97-101.

5. Press E, Porter RR, Cebra J. Isolation and properties of a proteolytic enzyme, cathepsin D, from bovine spleen. *Biochem J*, 1960; 74: 501-14.
6. Skrzydlewska E. Influence of ethanol and acetaldehyde on activity of the stomach proteolytic enzymes. *Ann Acad Med Bialostocensis*, 1986/1987; 31/32: 3-18.
7. Skrzydlewska E. Influence of ethanol and acetaldehyde on the activity of proteolytic enzymes of the small intestine and pancreas. *Ann Acad Med Bialostocensis*, 1986/1987; 31/32: 19-28.
8. Skrzydlewska E, Worowski K. Influence of ethanol and acetaldehyde on activity and release of cathepsins from lysosomes of the dog liver (studies in vitro). *Ann Acad Med Bialostocensis*, 1984/1985; 23/30: 59-76.
9. Worowski K, Ostrowska H. Cathepsin D. *Post Biol Kom*, 1980; 7: 119-47.
10. Zakrzewska I, Worowski K, Skrzydlewska E, Prokopowicz J, Puchalski Z, Piotrowski Z. Effect of ethanol and acetaldehyde on the enzymatic activity of human pancreatic juice in vitro. II. Inhibition of the activity of proteolytic enzymes. *Acta Med Pol*, 1988; 29: 47-52.