Stimulation of Pancreatic Lipase Activity by Saponins Isolated from *Medicago sativa* L.

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Saponins isolated from *Medicago sativa* L. appeared to stimulate lipolytic activity and did not influence amylolytic or proteolytic activity of Neopancreatinum (extract of pancreatic enzymes: lipase, proteases and amylases). Saponins isolated from the aerial part of *Medicago sativa* L. were demonstrated to stimulate lipase activity more effectively than saponins separated from the root of this plant.

When saponins were treated with 0.02 M HCl at 37 °C for 1 hour their lipase stimulation ability did not change. The saponins stimulated Neopancreatinum lipolytic activity more intensively in the presence of sodium cholate.

Introduction

Saponins are natural plant compounds with various biological activities such as: antiviral, antibacterial, antifungal, antiinsecticidal (Reznicek and Jurenitsch, 1991) and phytotoxic activity (Price *et al.,* 1987; Oleszek *et al.,* 1992a). The common feature of saponins is their surface activity.

Lipases are enzymes very sensitive to the presence of surface active compounds. They are inhibited by strong synthetic surfactants such as Triton X-100, SDS (Sroka, 1993; Gargouri *et al.,* 1989) or stimulated by weaker surfactants which are the natural effectors of this group of enzymes (bile salts sodium taurocholate or sodium cholate) (Sroka, 1993).

The main objective of these experiments is to test the effect of sodium cholate (CH) and saponins isolated from the root part (RP) and aerial part (AP) of alfalfa (*Medicago sativa* L.) var. Radius on lipolytic activity of Neopancreatinum (NP) which is a mixture of porcine pancreatic enzymes such as: lipase (27 F.I.P/1 mg of extract), amylase (32 F.I.P/1 mg) and proteases (trypsine, chymotrypsine 1.7 F.I.P/1 mg). The Neopancreatinum was obtained from Jelfa S. A. Pharmaceutical Works, Jelenia Góra, Poland.

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Material and Methods

Isolation of alfalfa saponins

Saponins were obtained from roots and aerial parts of alfalfa using the previously described methods (Oleszek *et al.,* 1990; Oleszek *et al.,* 1992b).

Measurement of Neopancreatinum lipolytic activity

The influence of intact saponins and treated with 0.02 M HCl at 37 °C for 1 hour on lipolytic activity of NP was measured by two methods:

a) the one method useful for measurement of pancreatic lipase activity during medical investigation described by Tietz and Fiereck (1966) with arabic gum as an emulsion stabiliser

b) the other method in which saponins were used as the only factor of emulsion stabiliser.

ad a) 2.5 ml of saponin solution (CH or CH with Saponins) in 0.043 M Tris-HCl buffer pH 8.0 at various concentrations was added to the test tubes. After that, 2.5 ml of olive oil emulsion and 1 ml of 0.2 M Tris-HCl buffer pH 8.0 was added. After 10 minutes of preincubation (37 °C) 1 ml of NP solution in water (15.1 mg/ml) was added. Reaction was stopped by adding 3 ml of 96% ethanol solu-

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tion after 6 h of incubation. Free fatty acids were measured by titration of samples with 0.05 N NaOH and phenolphthalein as an indicator.

Control samples were prepared in a similar way, but NP solution was added after ethanol addition. NP lipolytic activity stimulation was expressed by the S value calculated according to the following equation: $^{Vp}/_{Vo} \times 100 = S$; Vp - ml of 0.05 N NaOH used for titration of samples with saponins; Vo - ml of 0.05 N NaOH used for samples without saponins.

ad b) 18 ml of saponin solutions in 0.07 M Tris (hydroxymethyl)aminomethane – HCl buffer pH 8.0 at various concentrations were added to a round-bottomed flask (50 ml). Then 2 ml of olive oil was added to each sample. Flasks were vigorously shacked for 5 min. Emulsions were placed into Erlenmeyer flasks (50 ml) and heated in a water bath of 37 °C. After 10 minutes, 2 ml of NP solution (22 mg/ml) in 0.07 M Tris-HCl pH 8.0 was added. Free fatty acids were measured after 3 and 6 hours by withdrawing 10 - ml samples which were titrated with 0.05 N NaOH with phenolphthalein as indicator. Control samples were prepared in a similar way, but NP was added after ethanol addition.

The influence of saponins on proteolytic activity of Neopancreatinum

The proteolytic activity of NP in the presence of saponins was measured by the method of Kunitz *et al.* (1947). 0.5 ml of saponin solution at concentration of 0.12, 0.25, 0.38, 0.5 and 1 mg/ml in 0.2 M phosphate buffer (Na_3PO_4–NaH_2PO_4), pH 7.4 were added to the test tubes. Then 1 ml of 1% solution of casein in 0.1 M phosphate buffer, pH 7.4 was added. All samples were preincubated for 10 minutes at 37 °C. Then 0.5 ml NP solution in water (8 mg/ml) was added. Reaction was stopped after 30 min. by adding 3 ml of 5% trichloroacetic acid (TCA) in water. Samples were centrifuged (1000×*g*) and absorbance λ A_{280} of supernatant was measured in a 1 cm glass cuvette.

The influence of saponins on amylolytic activity of Neopancreatinum

The amylolytic activity of NP was measured by the method of Krawczyński *et al.* (1967) with modification.

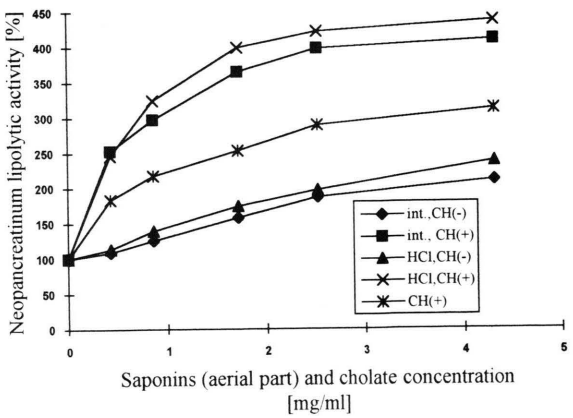
2.4 ml of substrate solution (0.5 g of starch, 1 g NaCl, 2 g sodium citrate, 100 ml water) and 0.05 ml of saponin solution in water at 1.4, 5.8, 23, 94, 375, 1500 μg/ml was added to the test tubes. Samples were preincubated 10 minutes at 37 °C. Then 0.05 ml NP solution in water at 0.125 mg/ml was added. After 30 minutes of incubation, at 37 °C reaction was stopped with 2.5 ml of 20% (in water) sulfosalicylic acid. In the case of control samples, NP solution was added after sulfosalicylic acid. Finally 0.5 ml of each sample was added to 0.5 ml of iodine solution (2 g KJ, 1 g J_2, 300 ml water). Absorbance λ 560 was measured in 1 cm glass cuvette.

Results

Influence of intact saponins and treated with 0.02 M HCl at 37 °C for 1 h and cholate on lipolytic activity of Neopancreatinum

Effects of saponin mixtures on lipolytic activity of NP are shown in Figs 1 and 2.

When saponins from AP of alfalfa were used the stimulating effect of NP lipolytic activity was observed. Lipolytic activity of NP was equal to 212 and 239% of control test (without saponins) in the presence of intact saponins and hydrolysed respectively at 4.31 mg/ml in reaction mixture. The stim-



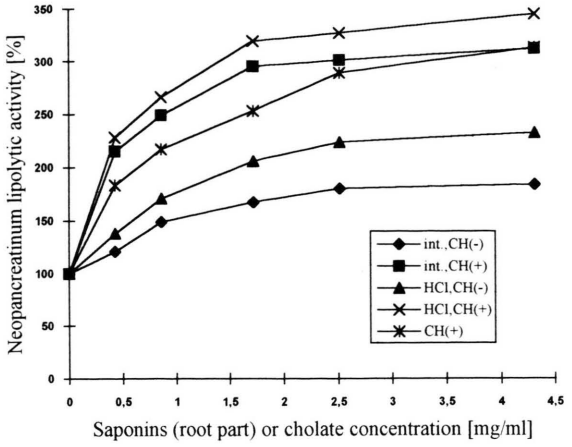
int. - intact saponins

HCl - HCl trated saponins

CH(-) - cholate absent

CH(+) - cholate present

Fig. 1. Effect of saponins from aerial part of alfalfa and cholate on lipolytic activity of Neopancreatinum.



int. - intact saponins

HCl - HCl trated saponins

CH(-) - cholate absent

CH(+) - cholate present

Fig. 2. Effect of saponins from root of alfalfa and cholate on lipolytic activity of Neopancreatinum.

ulating effect was much higher when saponins and CH were added to the reaction mixture simultaneously. In the presence of mixtures of intact saponins – CH or hydrolysed saponins – CH AT 4.31 mg/ml, NP lipolytic activity was equal to 412 and 439% of control test (without saponins) respectively Fig. 1.

When saponins from RP of alfalfa were used a lower stimulating effect in comparison with AP influence was observed (Fig. 2). In the presence of intact and hydrolysed saponins at 4.31 mg/ml NP lipolytic activity was equal to 184 and 233% respectively of control test activity. When mixtures of intact saponins – CH and hydrolysed saponins – CH were added simultaneously at 4.31 mg/ml, the NP lipolytic activity was 313 and 346% of control test respectively.

The influence of saponins from aerial part and cholate on lipolytic activity of Neopancreatinum when saponins and cholate were the only agents stabilising the emulsion

The effects of saponins, CH and mixture (saponins + CH) on NP lipolytic activity after 3 hours and after 6 hours of incubation is shown in Table I.

When saponins, CH and mixture were added to the tests at 4.5 mg/ml lipid hydrolysis by NP was equal to 150, 149 and 177% of controls respectively after 3 hours of incubation. The measurement of free fatty acids after 6 hours showed that in the presence of sabonins, CH and mixture reaction rate were equal to 173, 213 and 242%.

The influence of saponins from root part of alfalfa on lipolytic activity of Neopancreatinum when saponins and cholate were the only agents stabilising the emulsion

The effects of saponins and CH on NP lipolytic activity after 3 and after 6 hours of incubation is shown in Table I.

Saponins isolated from RP of alfalfa stimulated NP lipolytic activity. Amount of hydrolysed fatty acids by NP after 3 and 6 hours of incubation in the presence of saponins at 4.5 mg/ml were 147 and 178% of controls respectively. In the presence

Table I. Influence of saponins (root, aerial part of alfalfa) and cholate on lipolytic activity of Neopancreatinum (NP).

Effector concentration [mg/ml]	Enzyme activity (%) after 3 hours	Enzyme activity (%) after 6 hours
NP + Cholate		
0	100	100
1.818	127	158
4.545	149	213
NP + Saponin (aerial part)		
0	100	100
1.818	130	150
4.545	150	173
NP + Mixture (saponins from aerial part and cholate)		
0	100	100
1.818	144	180
4.545	177	242
NP + Saponins (root part)		
0	100	100
1.818	127	138
4.545	147	178
NP + Mixture (saponins – root part and cholate)		
0	100	100
1.818	138	214
4.545	148	220

of the mixture lipid hydrolysis was 148 and 220% of controls after 3 and 6 hours.

Influence of saponins from root part and aerial part of alfalfa on proteolytic activity of Neopancreatinum

Effects of saponin mixtures on proteolytic activity of NP is shown in Table II.

The main aim of these experiments was to test the effect of saponins on proteases: trypsin, chymotrypsin, enzymes being ingredients of NP. No important effects were observed in the range of concentration of saponins equalling to 0–2.5 mg/ml.

The influence of saponins on amylolytic activity of Neopancreatinum

The effect of saponins (from RP and AP) on amylolytic activity of NP is shown in Table II.

The aim of this experiment was to check the influence of saponins on amylolytic activity of NP. Only a very weak decrease of amylolytic activity was observed in a high range of concentration of saponins.

Table II. Influence of saponins from root and aerial part of alfalfa on proteolytic and amylolytic activity of Neopancreatinum (NP).

Saponin concentration [mg/ml]	Enzyme activity (%)
NP + Saponins (aerial part) proteolytic activity	
0.0	100
1.0	106
1.5	103
2.5	103
NP + Saponins (root) proteolytic activity	
0.0	100
0.5	114
1.5	131
2.5	118
NP + Saponins (aerial part) amylolytic activity	
0.000	100
0.094	102
0.375	102
1.500	81
NP + Saponins (root part) amylolytic activity	
Saponin concentration [mg/ml]	NP amylolytic activity (%)
0.000	100
0.094	89
0.375	89
1.500	73

Discussion

Results presented in this paper show that saponins isolated from alfalfa stimulated lipolytic activity and did not influence the proteolytic and amylolytic activity of Neopancreatinum which is an extract of the pancreas enzymes (trypsin, chymotrypsin, lipase and amylase).

It was demonstrated that surface active compounds had a varied and strong influence on lipases activity (Gargouri *et al.*, 1980; Sroka, 1993). Synthetic surfactants with high surface activity usually inhibited lipase activity (Sroka, 1993), but natural surfactants such as bile salts, with weaker surface activity, stimulated lipid hydrolysis in a wide range of concentration. Saponins isolated rom alfalfa are surfactants, but their amphiphilic properties have not been investigated yet.

This work shows that saponins from alfalfa stimulate lipolytic activity of Neopancreatinum. Much stronger stimulatory effect of saponins was observed when sodium cholate was added simultaneously. Incubation of saponins with HCl at 37 °C did not influence their stimulatory properties.

One can consider alfalfa saponins as possible medication which could be used as a substitution for natural bile salts in some diseases but, for this purpose, much more further clinical investigation is required.

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