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Jack bean urease inhibition by crude juices of *Allium* and *Brassica* plants. Determination of thiosulfinates

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ABSTRACT

The aim of this study was the elucidation of the inhibitory influence of *Allium* (garlic, onion, leek) and *Brassica* (cabbage, Brussels sprouts) plants juices, on jack bean urease activity. Concentrations of thiosulfinates, the compounds responsible for the inhibition, were determined in studied materials. The kinetics and mechanism of the inhibitions were investigated. Biphasic, time-dependent courses of the inhibition reactions were observed for all tested *Allium* and Brussels sprouts from *Brassica*. The cabbage material caused the monophasic course of the inhibition. In the presence of dithiothreitol, a total reactivation of the inhibited urease proceeded for the tested plants except for the onion. The onion juice modified urease, regained only half of the initial activity. The irreversible contribution was related to the presence of 1-propanethial-*S*-oxide, cepaenes and zwiebelanes formed in the onion juice. It was found that the thermal processing of the plant juices, results in the decrease of thiosulfinates concentration, as well as the efficiency of urease inhibition.

1. Introduction

Urease (urea amidohydrolase, EC 3.5.1.5.) is a nickel metalloenzyme that catalyses the hydrolysis of urea into carbon dioxide and ammonia:

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

Urease is an enzyme widely found in nature. It has been isolated from a variety of organisms, including yeast, fungi, algae, bacteria and several higher plants. All ureases show amino acid sequence similarity. However, in plants and fungi, the urease molecules are homohexamers while bacterial ureases, are multimers of two- or three-subunits complexes. Each monomer contains a bi-nickel active site directly involved in binding of substrates and inhibitors. The supporting role in the catalytic mechanism plays a cysteine residue situated in the side amino acid chain opening and closing the entrance to the active site. The modification of this essential cysteine strongly influences the catalytic activity of the enzyme. Structures of the active site are similar for all ureases, independently of the origin (Balasubramanian & Ponnuraj, 2010; Follmer, 2008; Karplus & Pearson, 1997; Mobley, Island, & Hausinger, 1995). Therefore, plant urease can serve as a model of urease for inhibitory studies.

Many bacteria producing urease are responsible for urinary tract infections e.g. *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp. (Mobley et al., 1995). *Helicobacter pylori* cause development of gastric, peptic and duodenal ulcers as well as gastric cancer. Unfortunately, *Helicobacter pylori* is a bacteria resistant to many antibiotics (Aguemon et al., 2005). Therefore, a lot of attention is being focused on searching for substances which are both highly effective in protection against *Helicobacter pylori* and safe for the gastrointestinal tract. The potent source of the aforementioned substances can be plants used as foodstuffs. Plants such as garlic (*Allium sativum*), onion (*Allium cepa*) and cabbage (*Brassica oleracea var. capitata*) were among the earliest cultivated foods and remedies used in folk medicine. Their chara cteristic flavour and antibacterial, antifungal and antioxidant properties originate in alk(en)yl thiosulfinates (TS) formed from precursors, alk(en)yl-L-cysteine sulfoxides (alk(en)yl-CSO) in the enzymatic reactions following tissue destruction (Arnault, Mondy, Diwo, & Auger, 2004; Block et al., 1996; Jones et al., 2004; Kyung & Fleming, 1994):

$$\begin{aligned} &R{-}S(O){-}CH_2{-}CH(NH_2){-}COOH + H_2O \xrightarrow{\text{allinase}} R{-}SOH \\ &\quad + CH_3{-}C(O){-}COOH + NH_3 2R{-}SOH \\ &\quad \rightarrow R{-}S(O){-}S{-}R + H_2O \end{aligned} \quad (1)$$

where R is methyl, 1-propenyl, 2-propenyl or propyl group.

At the initial period of the reaction, pyruvate, ammonia and alk(en)yl sulfenic acid R–SOH are formed. Afterwards R–SOH undergoes rapid transformation into alk(en)yl thiosulfinate R–S(O)–S–R.

There are four odourless alk(en)yl-CSO present in *Allium*: alliin (2-propenyl-CSO), isoalliin (1-propenyl-CSO), methiin (methyl-CSO) and propiin (propyl-CSO), but only methiin occurs in the

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intact *Brassica* plants. Isoalliin, methiin and propiin occur in the tissues of onion. Garlic contains alliin, methiin and propiin and in leek isoalliin and methiin are present (Jones et al., 2004; Kyung & Lee, 2001). (Shen, Hong, & Parkin, 2002; Shen & Parkin, 2000) studied the pathway of TS production. The authors found that the rate of enzymatic hydrolysis (Eq. (1)) of the alk(en)yl-CSO follows the order: isoalliin > alliin > propiin > methiin. This explains the composition of TS in *Allium* and *Brassica* tissues.

TS can decompose and form related organosulphur compounds. In garlic alliin is predominant alk(en)yl-L-cysteine sulfoxide, a precursor of the intermediate 2-propenyl sulfenic acid. This acid rapidly reacts and forms allyl-2-propenethiosulfinate (allicin) that subsequently degrades into di-2-propenyl disulfide, thiosulfonate, polysulfides and ajoen (Rose, Whiteman, Moore, & Zhu, 2005). Isoalliin is dominant in onion and it is the precursor of the intermediate sulfenic 1-propenyl acid which forms TS as well as cepaenes, zwiebelanes and propanethial-*S*-oxide, named lachrymatory factor (LF) (Arnault et al., 2004; Benkeblia & Lanzotti, 2007; Block, Dane, Thomas, & Cody, 2010). LF is the compound responsible for inducing tears, while cutting onion. The *Brassica* species contain only methiin, which is converted into methylmethanethiosulfinate and then to dimethylthiosulfonate, dimethylsulfide and polymethylsulfides (Kyung & Lee, 2001; Rose et al., 2005). The inhibition of jack bean urease by garlic extract has already been investigated by our staff (Juszkiewicz, Zaborska, Sepioł, Góra, & Zaborska, 2003; Juszkiewicz, Zaborska, Łaptaś, & Olech, 2004; Zaborska, Karcz, Kot, & Juszkiewicz, 2009). It has been found that allicin the most abundant TS present in fresh garlic extract is responsible for inhibition of the enzyme.

The aim of this work was to study the inhibition ability of other TS rich vegetables against urease. The inhibiting effects of *Allium* plants (onions and leek) as well as *Brassica* (cabbage and Brussels sprouts) were investigated. The content of thiosulfinates in studied materials was determined and the kinetics of inhibition elucidated. The results were compared with the data obtained for garlic, which was found to be very effective in blocking of urease. The influence of the thermal processing of the studied juices on the inhibition abilities against urease was examined. All studied vegetables were components of daily European diet. The present research evaluated the selected *Allium* and *Brassica* plants as a source of natural antibiotics against pathogenic bacteria.

2. Materials and methods

2.1. Materials

Jack bean urease, Sigma type III (specific activity 22 U/mg solid), urea (Molecular Biology Reagent), L-cysteine, dithiothreitol (DTT), 5.5′-dithiobis-(2-nitrobenzoic acid) (DTNB), were purchased from Sigma. All the remaining chemicals were of analytical grade. All vegetables: garlic (*Allium sativum*), yellow and white onion (*Allium cepa*), leek (*Allium porrum*), cabbage (*Brassica oleracea* var. *capitata*), Brussels sprouts (*Brassica oleracea* var. *gemmifera*) originating from traditional farming were purchased from local producers in south Poland. Randomized market sampling was applied. The average sample consisted of representative amounts of five individual samples from different farmers.

2.2. Preparation of plants juices

Garlic cloves, onions bulbs, leek and cabbage leaves, and Brussels sprouts buds were sliced and pulped. The squeezed juices were filtered through a gauze. Then the undissolved material was removed by centrifugation at 300*g* for 4 min. The dry matter content was determined by drying the samples at room temperature (22–24 °C) to constant weight which lasted about one week. Three samples for each plant were used.

2.3. Determination of thiosulfinate concentration

Thiosulfinate concentration was determined by applying a spectrophotometric method of Han et al. (Han, Lawson, Han, & Han, 1995). The method is based on the fact that one molecule of thiosulfinate reacts with two molecules of L-cysteine to form two molecules of S-alk(en)yl-mercaptocysteine. The decrease of L-cysteine content is measured spectrophotometrically at 412 nm in the form of 2-nitro-5-thiobenzoic acid (NTB) obtained in reaction with DTNB. The crude juice was mixed with 10 mM L-cysteine with the volume proportion of 1:1 and incubated for 10 min. Then 100 μM of the mixture was removed and added to 5 cm^3 of 0.15 mM DTNB. After a 10 min incubation the concentration of L-cysteine was determined indirectly by spectrophotometric measurement of the liberated NTB.

2.4. Standard urease activity assay

The standard assay mixture (25 cm^3) consisted of 50 mM urea in 20 mM phosphate buffer, pH 7.0 and 2 mM EDTA. The reactions were initiated by the addition of small aliquots of the enzyme-containing solution (0.5 cm^3) and the urease activity was determined by measuring ammonia concentration after a 5 min reaction. Ammonia was determined by the spectrophotometric, phenol-hypochlorite method. The absorbance was registered at 630 nm (Weatherburn, 1967). The measurements were performed at 22 °C. The activity of uninhibited urease was accounted as the control activity of 100%.

2.5. Inhibition studies

The urease solution was mixed with an equal volume of juice, and the mixture was incubated at 22 °C. The mixture always contained 0.5 mg/cm^3 of urease, 20 mM phosphate buffer, pH 7.0 and 2 mM EDTA. Aliquots were taken at different time intervals and were immediately transferred to the assay mixtures containing 50 mM urea, 20 mM phosphate buffer, pH 7.0 and 2 mM EDTA in order to determine the enzyme residual activity. Prior to the enzymatic reaction, ammonia concentration in the juice produced during thiosulfinate formation (Eq. (1)) was determined. This amount was subtracted from the total amount of ammonia in the system.

2.6. Reactivation of urease in the presence of DTT

The reactivation of the crude juices inactivated urease was studied by using DTT. The urease solution was mixed with an equal volume of the crude juice and incubated. The incubation mixture contained 0.5 mg/cm^3 urease, the crude juice, 20 mM phosphate buffer, pH 7.0 and 2 mM EDTA. Urease was incubated with the tested juice until urease lost approximately 90% of its activity. Then a small volume of DTT solution was added. The DTT concentration in the incubation mixture was equal to 10 μM i.e. 10-fold higher than the TS concentration in the tested juice. The activity of urease was determined before and after DTT addition. After appropriate period of time, samples of the incubation mixture were withdrawn and transferred into the standard assay mixture and urease residual activity was determined.

2.7. Thermal treatment of the vegetable juices

The vegetable juice was poured into 6 test-tubes, each with the capacity of 5 cm^3. The test-tubes with solutions were heated in a