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Effect of spermidine and its metabolites on the activity of pea seedlings diamine oxidase and the problems of biosensing of biogenic amines with this enzyme

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ABSTRACT

Spermidine is one of the several biogenic amines, produced during the microbial decarboxylation of proteins. Individual biogenic amines in the formed mixtures are frequently analyzed with oxygen sensor based biosensors, as their content serves as a good biomarker for the determination of food quality. In these biosensors, diamine oxidase from pea seedlings (PSAO), catalyzing the oxidation of various biogenic amines by dissolved oxygen is commonly used for the bio-recognition of amines. However, in the presence of spermidine and/or its metabolite 1,3-diaminopropane, the activity of PSAO and the sensitivity of PSAO-based biosensors decrease due to inhibition. The inhibition constant of soluble spermidine, acting as an inhibiting substrate toward PSAO, was found to be (40 ± 15) mM in freshly prepared solution and (0.28 ± 0.05) mM in solution, incubated 30 days at room temperature. The inhibition constant of 1,3-diaminopropane, acting as a competitive inhibitor, was (0.43 ± 0.12) mM as determined through the oxidation reaction of cadaverine. The metabolic half-life of soluble spermidine was 7 days at room temperature and 186 days at 4 °C. The kinetic measurements were carried out with an oxygen sensor; the composition of the solution of degraded spermidine was analyzed with MS.

1. Introduction

Biogenic amines are biologically active low molecular weight compounds, formed by microbial decarboxylation of protein-containing material from the corresponding amino acids or by transamination of aldehydes and ketones, catalyzed by amino acid transaminases (EC 2.6.1.21) [1]. Putrescine (1,4-diaminobutane), cadaverine (1,5-diaminopentane) and spermidine (*N*-(3-aminopropyl)-1,4-butanediamine), also known as biogenic polyamines, are primarily the products of microbial activity [2] and used to serve as natural biomarkers for the determination of food quality [3–7]. The preliminary compounds of the formation of biogenic amines are basic amino acids—arginine, histidine and lysine, and ornithine, formed from arginine in urea cycle. Both arginine and ornithine, which are decarboxylated by arginine decarboxylase (EC 4.1.1.19) and ornithine decarboxylase (EC 4.1.1.17) respectively, are turned into putrescine [8,9], which can be further converted into spermidine. The well-characterized ornithine decarboxylase is specific toward ornithine and does not produce cadaverine from lysine, although ornithine and lysine are structurally very closely related as their decarboxylation products putrescine and cadaverine [10]. Lysine is converted into cadaverine by lysine decarboxylase (EC 4.1.1.18) [10]. Besides polyamines, the most common biogenic amine is histamine, product of histidine decarboxylation (EC 4.1.1.22) [11]. The putrefaction of proteins, macromolecules consisting of the residues of different amino acids, leads to the formation of different mixtures of biogenic amines, depending on the protein content.

Diamine oxidase (EC 1.4.3.22) is a Cu^{2+}-dependent enzyme which catalyzes the oxidative deamination of different amines and is widely used for the bio-recognition of biogenic amines in biosensors. The overall deamination reaction, actually following the ping-pong mechanism [12–14], can be presented as follows:

$$R - CH_2NH_3^+ + O_2 + H_2O \rightarrow NH_4^+ + R - CHO + H_2O_2 \quad (1)$$

The activity of pea seedlings diamine oxidase (PSAO) toward different biogenic amines varies within a wide range [15–18]. Data about the substrate specificity of PSAO, presented by different authors, is variable and depends on the applied experimental method [7]. The sensitivity of PSAO is the highest toward symmetric diamines—cadaverine and putrescine. In addition to these two amines, a noticeable activity has been found toward spermidine;

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Table 1
Substrate specificity of pea seedling diamine oxidase.

Biogenic amine	Specific activity of PSAO (%)	Detection system	Ref.
Cadaverine	100	Electrochemical sensor: enzyme modified graphite electrode	[19]
Putrescine	80		
Spermidine	24		
Histamine	11		
Cadaverine	100	Electrochemical sensor: enzyme modified carbon paste/graphite powder electrode	[20]
Putrescine	96		
Spermidine	17		
Histamine	9		
Cadaverine	100	Fiber optical sensor: enzyme modified magnetic chitosan microparticles	[21]
Putrescine	117		
Spermidine	19		
Histamine	13		
Cadaverine	100	Fiber optical sensor: enzyme modified SEPABEADS® EC-HA 403	[21]
Putrescine	126		
Spermidine	56		
Histamine	17		
Cadaverine	100	Oxygen sensor	[22]
Putrescine	86		
Histamine	10		

the activity toward histamine is much lower [19–22]. A condensed overview of substrate specificity of PSAO is presented in Table 1.

Most biogenic amines are stable, except spermidine, which undergoes quick metabolic changes. Ruiz-Capillas and Moral have studied the changes of biogenic amines concentrations in fish samples during storage in different atmosphere [23]. They noticed that the concentrations of all studied biogenic amines increased, but the concentration of spermidine fluctuated in time. A similar fluctuation has been reported in other studies [24–27]. This phenomenon can be the result of the action of microorganisms [28,29] or the spermidine non-catalytic oxidation [18].

The oxidation of spermidine by molecular oxygen initially yields 1,3-diaminopropane (1,3-DAP) and 4-aminobutyraldehyde; the latter undergoes rapid spontaneous intramolecular Schiff-base formation to yield Δ^1-pyrroline [30]:

$$\text{Spermidine} + \frac{1}{2}O_2 \rightarrow \text{1,3-diaminopropane} + \text{4-aminobutyraldehyde} \underset{\Delta^1 - \text{Pyrroline}}{\downarrow} \quad (2)$$

Δ^1-Pyrroline formation is also reported during the oxidation of putrescine, which similarly to spermidine is initially converted into γ-aminobutyraldehyde further forming Δ^1-pyrroline [31]. The essential difference between spermidine and putrescine oxidation is the formation of 1,3-diaminopropane from spermidine.

It has been shown that diamine oxidase from plants has no catalytic activity toward 1,3-diaminopropane [32]. On the contrary, 1,3-diaminopropane can act as an inhibitor for this enzyme [33–36]. So, the formed 1,3-diaminopropane influences the detection of biogenic amines in real samples, like food and beverages with PSAO-based biosensor.

The aim of the present study was to characterize the potential inhibitory effect of spermidine and its metabolite 1,3-diaminopropane on the activity of pea seedlings diamine oxidase and the biosensing of individual biogenic amines or their total concentration in mixtures, forming during putrefaction of proteins. The catalytic activity of PSAO and the sensitivity of PSAO-based biosensors change in the course of measurements of biogenic amines due to the irreversible deamination of spermidine. We used oxygen sensor to follow the PSAO-catalyzed oxidation reactions; the spermidine decay was additionally analyzed with MS.

2. Materials and methods

2.1. Chemicals and reagents

Spermidine (SPD), 1,3-diaminopropane (1,3-DAP) and cadaverine (CAD) standards were purchased from Sigma–Aldrich (Germany). Diamine oxidase was isolated and purified from pea (*Pisum sativum*) seedlings as described in [32]. PSAO activity in the enzyme extract was 12.0 U/ml. The extract was stored at −20 °C and used within 4 h after melting at 4 °C.

All chemicals were of analytical grade unless otherwise stated. All aqueous solutions were prepared with ultrapure DI water (18.2 MΩ/cm).

2.2. Experimental procedures using biosensing system

The change of dissolved oxygen concentration in reaction medium due to the oxidation of biogenic amines was followed with a Clark-type oxygen sensor (covered with 25 μm thick polyethylene film with the area of 5.65 cm^2, *Elke Sensor* LLC, Estonia). All experiments were carried out under constant stirring in air-saturated 0.1 M phosphate buffer solutions (pH 7.00, in DI water) at 25.0 °C in thermostated air-tight reaction cell. The sensor was put into the mixture of biogenic amines and the reaction was started by injection of PSAO extract; the final PSAO concentration in the reaction medium was always 0.043 U/ml. The sensor signal was registered automatically with 0.5 s interval; the obtained data were normalized and used for the calculation of reaction parameters.

2.3. Calculation of reaction parameters

The reaction characteristic parameters were calculated using the dynamic model for biosensors [37]. This model enables to calculate the reaction kinetic and steady-state parameters from the transient phase response, minimizing the influence of side processes (H_2O_2 degradation, oxygen mass transfer through the liquid–air surface etc.) and avoiding the uncertainty of determining the steady state. The calculated steady state parameter is depending hyperbolically on amine concentration.

2.4. Mass spectrometric measurements

The measurements were carried out using an Agilent XCT ion trap mass spectrometer equipped with electrospray interface (ESI). For infusion experiments, solutions were directly infused into mass spectrometer with syringe pump at flow rate 0.5 ml/h. Spectra was collected for 1 min and averaged. ESI source parameters were: nebulizer gas (nitrogen) pressure 50 psi (345 kPa), drying gas (nitrogen) flow rate 12 l/min and drying gas temperature 350 °C.

3. Results and discussion

The activity of PSAO toward studied amines was characterized with the calculated normalized total signal change parameter *A*, corresponding to the maximum signal change at steady state conditions [38]. The dependence of this parameter on the concentration of spermidine is presented in Fig. 1. This dependence had an irregular bell-shape form with a flat maximum at spermidine