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VELLOSIMINE REDUCTASE, A SPECIFIC ENZYME INVOLVED IN THE CELL-FREE BIOSYNTHESIS OF SARPAGINE TYPE ALKALOIDS

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Abstract: Vellosimine reductase is a substrate and cofactor specific enzyme which catalyzes the NADPH-dependent reduction of vellosimine (4) forming 10-deoxy-sarpagine (5) in the cell-free biosynthesis of sarpagine type alkaloids.

Recently we investigated the cell-free conversion of polyneuridine aldehyde (1) into 16-epi-vellosimine(3) and vellosimine (4), a key sequence in the biosynthesis of sarpagine type alkaloids [1]. The formation of 16-epi-vellosimine (3) is catalyzed by polyneuridine aldehyde esterase (PNA-esterase), an enzyme exhibiting an exceptional high substrate specificity. The esterase generates the sarpagine-characteristic monoterpenoid C_9-unit by a hydrolysis and decarboxylation step at the stage of polyneuridine aldehyde (1) which exhibits the monoterpenoid C_{10}-part. This enzyme thus provides the biogenetic precursor (4) for "late stage" reactions (reduction, hydroxylation) leading to sarpagine (6).

In this paper we report on the isolation and catalytic properties of the next enzyme in the pathway; vellosimine reductase. This enzyme converts vellosimine (4) into 10-deoxy-sarpagine (5) which might serve as the immediate precursor for sarpagine (6) (scheme).

As previously demonstrated, cell suspension cultures of Rauwolfia serpentina Benth. produce mainly ajmaline/sarpagine type alkaloids [2]. Therefore we used these cultured cells as an enzyme source for the investigation of the cell-free reduction of (4). In a typical procedure we obtained a crude enzyme mixture by extraction of the crushed cells with 50mM phosphate buffer (pH 7.0), protein precipitation between 10 - 70% $AmSO_4$ and gelfiltration (G-25). 3 ml (30 mg protein) of the enzyme mixture were incubated in 10 ml 0,1 M phosphate buffer (pH 7.5) containing 10 mg (12 µmol) NADPH and 0,22 mg (0,75 µmol) {aryl-^{3}H}-vellosimine (4), prepared from {aryl-^{3}H}-polyneuridine aldehyde (1) and PNA-esterase. After an incubation time of 2 hours at 30°C, the mixture was extracted with ethyl acetate. The organic phase was analysed by t.l.c. showing that one compound was formed in a yield of about 90%. The formation of this product was entirely dependent on the presence of NADPH indicating the reduction of (4). For a "large scale" preparation of this cell-free formed alkaloid and for unambiguous structure elucidation 140 mg enzyme were incubated under the above

Polyneuridine aldehyde (1) → [Polyneuridine aldehyde acid (2)] → 16-epi-Vellosimine (3) →

Vellosimine (4) → 10-De oxy-sarpagine (5) → Sarpagine (6)

Scheme

condition in presence of 12 mg vellosimine (4) and an NADPH regenerating system (190 mg $NADP^+$, 2,8 ml ethanol, 5 mg alcohol dehydrogenase from <u>Leuconostoc mesenteroides</u>, EC 1.1.1.2) in a tot. vol. of 350 ml. From this mixture 8 mg (yield 66%) of the unknown compound($\{\alpha\}_D^{20}$= +42°, c=0,51,MeOH) were isolated after purification on t.l.c. with $CHCl_3$:MeOH:NH_3 = 90:10:0,1. Mass spectroscopic and ^{1}H-NMR data of the cell-free reduced compound revealed : 294 (M^+, 53), 293 (M^+-H, 30), 263 (M^+-CH_2OH, 16), 249 (6), 182 (11), 169 (100), 168 (74), 115 (22) m/z, monoacetate M^+ 336 m/z; ^{1}H-NMR in acetone-D_6: 5.39 (C=C$\underline{H}$-CH_3, q, J=7 Hz, fine coupling), 4.16 (H-3, dd, J_1=10 Hz, J_2=2,5 Hz), 3.49 (1H, -C$\underline{H}_2$OH, dd, J_1=10.5 Hz, J_2=6 Hz), 3.42 (1H, -C$\underline{H}_2$OH, dd, J_1=10.5 Hz, J_2=8.5 Hz), 1.60 (3H, C$\underline{H}_3$-CH=, dt, J_1=7 Hz, J_2=2 Hz) ppm. These data demonstrate the reduction of the aldehyde group of (4) and are also in complete agreement with the published spectroscopic values for 10-desoxy-sarpagine (5) (<u>syn</u>. vellosimol, nor-macusine-B, desformo-akuammidinol, tombozine)[3-7] as well as with data obtained from the BH_4^- reduction product of (4).

This new plant enzyme involved in the biosynthesis of sarpagine-type alkaloids by catalyzing the reduction of (4), we have named vellosimine reductase. To characterize the reductase in more detail the enzyme was 42-fold purified by a 3-step procedure employing DEAE-cellulose-, hydroxylapatite- and Dye-ligand-chromatography (Matrex Gel Green A).At that stage of purification vellosimine reductase showed the following properties: an apparent molecular weight of 42000 ± 8%, an isoelectric point at pH 5.0, a pH optimum at pH 7,5 and normal Michaelis-Menten Kinetics for the substrate vellosimine (4) (K_m 16,7 μM, V_{max}

Substrate	Enzyme activity: rel. act. %	Enzyme activity: spec. act. (nkat/mg)	Substrate	Enzyme activity: rel. act. %	Enzyme activity: spec. act. (nkat/mg)
Vellosimine	100	(20)	Polyneuridine aldehyde	2.5	(0.5)
Geissoschizal	80	(16)	Picralinal	<0.5	(<0.1)
Anthirinal	2.3	(0.4)	Secologanin	<0.5	(<0.1)
Akuammidine aldehyde	3.0	(0.6)	2.5 - Dimethyl - benzaldehyde	<0.5	(<0.1)

Tab. 1: Substrate specificity of Vellosimine reductase

24 pkat). For the co-substrate NADPH an apparent K_m of 20 µM and V_{max} 28 pkat were determined. As it has been established for other aldehyde reductases isolated from plant tissue [8,9], the here described enzyme is also NADPH-specific (NADH, V_{max} 0). Moreover, the low K_m-value for vellosimine and investigations regarding the substrate specificity of vellosimine reductase point to a significant function of this enzyme in the biogenetic conversion of sarpagine type alkaloids. Of various alkaloidal and nitrogen-free aldehydes only few compounds are accepted as a substrate (Tab. 1). Besides the true substrate vellosimine, only compounds structurally closely related to vellosimine are reduced (e.g. geissoschizal → geissoschizol, K_m 110 µM, V_{max} 19 pkat) indicating a surprisingly specificity of the isolated reductase.

As the final step in the biosynthesis of sarpagine (6) the hydroxylation of (5) would be a plausible assumption. The Rauwolfia culture used for the experiments reported here synthesizes (6) and therefore we investigate at the present time this reaction at the cell-free level.

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