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NON-COMPETITIVE INHIBITION OF ENZYMATIC REACTIONS BY CANAVANINE

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Canavanine is known to be a competitve inhibitor of arginine utilization in animals, microorganisms, and higher plants [1,2,3]. But not in all experiments the canavanine inhibition of plant and plant tissue growth was reversed by arginine [4]. In former grafting and feeding experiments we were able to show the toxic effect of the amino acid an canavanine-free leguminous plants [5]. It was not possible to explain the inhibitory role of the compound in these experiments until now, because the arginine metabolism of the plants was not blocked competitively. Therefore a new unknown mechanism of canavanine action was supposed.

In some experiments with purified enzymes, not related to arginine metabolism, results were obtained now, showing a high non-competitive canavanine inhibition of alcohol dehydrogenase, ß-glucosidase, oxynitrilase, and several enzymes from different sources. The present publication deals with the observations about canavanine action on alcohol dehydrogenase activity.

Alcohol dehydrogenase (ADH) from yeast and NAD produced by Arzneimittelwerk Dresden (Fermognost Blutalkohol-Test) and canavanine · H_2SO_4 (L.Light & Co. Ltd., Colnbrook) were used. The enzyme action on ethyl alcohol was measured by the reduction of

NAD at pH 8,7 with an Eppendorf-photometer at 366 m μ. In each experiment 1,8 ml buffer pH 8,7 (containing 33,4 mg $Na_4P_2O_7$ · 1o H_2O, 8,o mg semicarbazide · HCl, 1,7 mg glycin, and 2,7 mg NaOH in 1 ml), 1oo μl NAD solution (1,5 mg NAD), 1o μl commercial ADH suspension (o,5 mg protein), and o,5 ml H_2O, o,5 ml arginine · H_2SO_4 solution ($5x1o^{-2}$ M), or o,5 ml canavanine · H_2SO_4 solution ($5x1o^{-2}$ M) were mixt. The reaction was started by adding 1oo μl substrate (2o μg).

Some of the results are summarised in Tab.1. They show that nearly the whole substrate is oxidized within 6 minutes (water control). The same results were obtained in the presence of arginine. But if arginine is substituted by canavanine, only about 3o% of the substrate were oxidized during the same time.

TAB. 1: Oxidation of Ethyl Alcohol by ADH and the Inhibition of this Reaction by Canavanine. Results are given in μg oxidized Substrate.

t in sec. / Addition of:	6o	12o	18o	24o	3oo	36o
H_2O	11,o	12,8	13,6	15,2	16,2	17,2
Arginine	1o,6	12,4	13,8	15,o	16,6	17,2
Canavanine	4,o	4,6	5,2	5,6	6,o	6,2

To study the enzyme action within the first 12o sec., the ADH concentration in the reaction mixture was reduced to 1/1o (5o μg ADH) or to 1/25 (2o μg ADH) in another series of experiments. The substrate was added in a concentration of 1oo μg, and the canavanine concentration was varied from $1x1o^{-4}$ to $1x1o^{-3}$ M. The results of these experiments are shown in Tab. 2.

The data presented here demonstrate a high inhibition of ADH action by canavanine. Analogous results were obtained when cana-

vanine was added to the reaction mixture of other purified plant enzymes. Arginine in the same concentration shows no effect, neither on the inhibited nor on the not inhibited reactions. Therefore the mechanism of action must be quite different from the hitherto known canavanine effects.

TAB. 2: Oxidation of Ethyl Alcohol by ADH and the Inhibition of this Reaction by different Canavanine Concentrations. Results are given in % Inhibition.

	5o µg ADH	2o µg ADH
Can ($1x1o^{-3}$ M)	47	34
Can ($1x1o^{-3}$ M) + Arg ($1x1o^{-3}$ M)	48	32
Can ($5x1o^{-4}$ M)	27	18
Can ($5x1o^{-4}$ M) + Arg ($5x1o^{-4}$ M)	25	2o
Can ($1x1o^{-4}$ M)	5	4

ADH from yeast is a tetramer and has a molecular weight of 15o.ooo. The active enzyme contains four moles of NAD and four atoms of zinc. Each individual chain contains a reactive SH group and, by binding one atom zinc and one mole NAD, is presumably capable of forming an independent "active centre" within the quarternary structure of the active tetramer. The removal of zinc for instance, causes the dissociation of the enzyme into inactive sub-units with a molecular weight of 36.ooo. This dissociation does not occur in the presence of NAD [6,7,8].

In some other experiments the enzyme (2o µg ADH) was pre-incubated with NAD or with canavanine ($5x1o^{-4}$ M) for five minutes. The data in Tab. 3 show that the pre-incubation with NAD has no significant effect on the inhibitory action of canavanine. Similar results were obtained when the enzyme was pre-incubated with NAD for five minutes and than with canavanine for five minutes.

In the contrary, pre-incubation with canavanine causes a stronger inhibition, and this effect was slightly reduced by a further pre-incubation with NAD for five minutes.

TAB. 3: Effect of Pre-incubation with NAD or with Canavanine ($5x10^{-4}$ M) for five Minutes on ADH Inhibition by Canavanine. Results are given in % Inhibition.

Pre-incubat. with: \ t in sec.	2o	4o	6o	8o
NAD	17,6	21,o	2o,8	2o,7
Canavanine	73,6	74,5	68,8	64,1
without Pre-incubation	16,6	16,8	16,1	17,1

These results suggest that the canavanine molecule may act in a manner of an allosteric inhibitor by blocking the formation of the active tetramer from the sub-units present in the enzyme solution. After adding NAD the active tetramer has been formed, than canavanine is less effective in inhibiting the enzyme action.

The highest canavanine concentration in the reaction mixtures nearly reaches the concentration present in the young plants of our grafting experiments with Canavalia ensiformis DC.[5]. Therefore some, if not all effects observed in these grafting experiments may find an explanation in the non-competitive canavanine inhibition. The results of a detailed kinetical study will be published elsewhere.

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