TRANSAMIDINASE OF HOG KIDNEY IV. EFFECT OF DINITROPHENYLATION°

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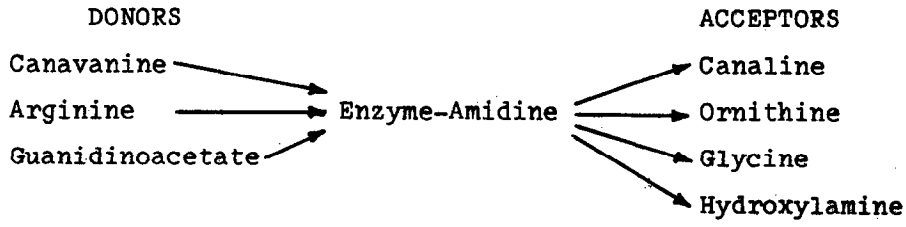
The transamidinase reaction can be depicted as follows (Walker, 1957) :



The enzyme-amidine complex has been isolated and its ability to transfer the amidine group to suitable acceptors has been shown (Grazi et al., 1965). Transamidinase catalyzes also an hydrolytic reaction in which the enzyme-amidine complex is split by water with the formation of free enzyme and urea (Ratner and Rochovansky, 1956; Conconi and Grazi, 1965). The treatment of the enzyme with p-hydroxymercuribenzoate leads to inactivation of both the hydrolytic (Vigi et al., 1965) and the transfer reaction (Walker, 1957). On the contrary, dinitrophenylation of transamidinase preferentially affects the transfer activity to glycine or hydro -

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xylamine while the hydrolytic reaction is slightly activated. This result is reported and discussed in the present paper.

METHODS

Transamidinase has been prepared as previously reported (Conconi and Grazi, 1965).

Dinitrophenylation - The incubation mixtures (0.45 ml) contained transamidinase (specific activity 70 units per mg of protein) 2 mg; borate buffer 0.2 M, pH 9; dinitrofluorobenzene either 50 or 100 mμmoles (2.5 or 5 moles per 100,000 gr of enzyme). The reaction was performed at 20° in the dark with stirring. A control sample, without dinitrofluorobenzene, was prepared and treated under the same conditions. After 40 minutes of incubation the protein of the samples was precipitated with 200 mg of ammonium sulfate, dissolved in 0.3 ml of phosphate buffer 0.015 M ,pH 7.5 and dialyzed for two hours at 2° against the same buffer.

Protein concentration was measured at 280 mμ or with the turbidimetric method of Bücher (1947). The method was calibrated by dry weight determinations on the dialyzed enzyme.

Enzymatic activity was determined following ornithine liberation with the colorimetric procedure of Chinard (1952).

The incubation mixtures contained 0.1 M phosphate buffer, pH 7.5; 0.01 M arginine; and either glycine 0.016 M or hydroxylamine 0.1 M. For the determination of the hydrolytic activity the acceptor substrate was omitted. The pH was 7.5 and the temperature 37°.

Arginine- ornithine transamidination was performed at 37° and pH 7.5. The incubation mixtures (0.25 ml) contained L-arginine U.L. ^{14}C (specific activity 225,000 cpm per micromole) 5.4×10^{-3}M; L-ornithine 5.2×10^{-3}M and 0.011 mg of transamidinase, either native or treated with 5 moles of fluorodinitrobenzene per mole of enzyme as already described. The reaction was started by addition of the enzyme. At various time, 0.05 ml

aliquots were taken, and the reaction was stopped by addition of 0.05 ml of 4 N HCl. The samples (containing approximately 60,000 cpm) were taken to driness, dissolved in 0.02 ml of water and chromatographed (descending) on Whatman N1 paper with the solvent system: phenol-water-ammonia 80:20:0.5 .The areas of radioactivity corresponding to arginine and to ornithine were eluted. On the resulting solutions the specific activity of ornithine was determined and expressed as cpm per micromole of ornithine.

RESULTS AND DISCUSSION

Fig. 1 shows that dinitrophenylation of transamidinase leads to inactivation of the transfer reaction to glycine and hydro-

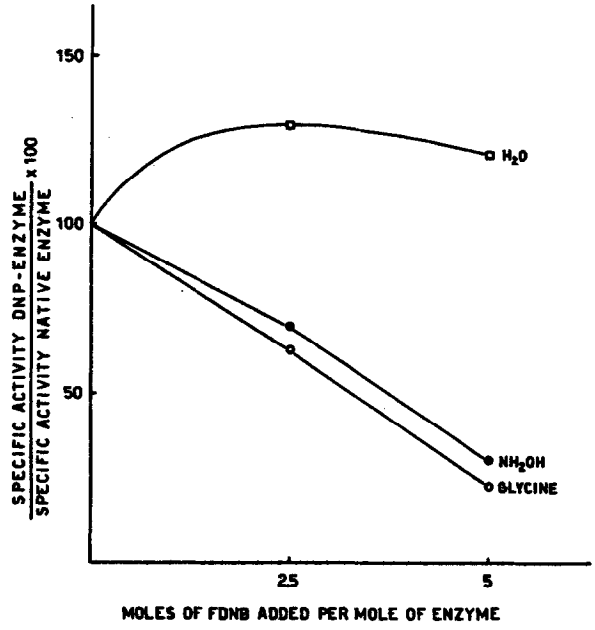

Fig. 1) Transamidinase activity and dinitrophenylation - The dinitrophenylated enzyme and the control sample have been prepared and assayed as described under Methods. On the ordinate is reported the ratio between the specific activity of the DNP-enzyme and the specific activity of the native enzyme. □—□ Hydrolytic reaction ●—● Transfer reaction with hydroxylamine ○—○ Transfer reaction with glycine.

xylamine. With 5 equivalents of fluorodinitrobenzene per mole of enzyme, after 40 minutes of incubation, the reactions with glycine and hydroxylamine are inactivated respectively of 78 and 70 %. Conversely the rate of the hydrolytic reaction is increased of 20 %.

The exchange reaction between arginine and ornithine is also inactivated to the extent of 60 % by the fluorodinitrobenzene treatment (Fig. 2).

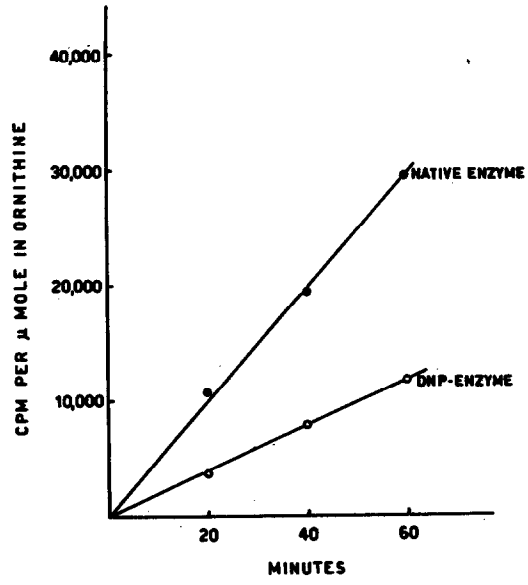

Fig. 2) Effect of dinitrophenylation on the arginine-ornithine exchange reaction - The experiments have been performed as described under Methods.

An immediate explanation of these results would be that dinitrophenylation affects the transfer reaction and not the formation of the enzyme-amidine complex. But this reasoning would be wrong since the hydrolytic reaction with the normal enzyme, being very slow, must occur with essentially all the enzyme

in the enzyme-amidine complex and, therefore, the rate of formation of the complex could be substantially decreased by dinitrophenylation without appreciably affecting its steady state level or, the rate of the hydrolytic reaction.

However, if dinitrophenylation affected the first step and not the second, the fact that the overall rate of the transfer reaction is lowered by dinitrophenylation must reflect a lower steady state level of enzyme-amidine complex at the usual glycine concentration.

Since the maximum possible level of enzyme-amidine complex (at zero glycine concentration) is the same for normal and DNP-enzyme (i.e. all the enzyme in the amidine complex), a decrease

Table I

Transamidinase and DNP-Transamidinase Activities as a Function of Glycine Concentration

Glycine Concentration	Ornithine Formed per Minute (μmoles)		Ratio
	Native Enzyme (1)	DNP-Enzyme (2)	(2)/(1)
16 $x10^{-3}M$	11.8	7.20	0.61
4 $x10^{-3}M$	9.6	5.70	0.51
1 $x10^{-3}M$	5.35	3.45	0.64
5 $x10^{-4}M$	3.45	2.31	0.67
2.5$x10^{-4}M$	2.47	1.62	0.65
1.25$x10^{-4}M$	1.48	1.04	0.70

The incubation mixtures (0.6 ml) contained 0.1 M phosphate buffer, pH 7.5; 0.01 M arginine; glycine as indicated and either 0.011 mg of native transamidinase or 0.022 mg of transamidinase treated with 5 equivalents of fluorodinitrobenzene. Temperature was 37°

in glycine concentration should result in a proportionately greater increase in the level of the complex with the DNP-en-

zyme that with the normal enzyme. This should be reflected in a ratio of activity of DNP-enzyme to normal enzyme which increases substantially with a decrease in glycine concentration. On the contrary the data of Table I show that the ratio remains essentially constant over a seven fold decrease of the rate of the reaction.

These results therefore show that the dinitrophenylation of the enzyme is likely to affect the transfer step. The data do not allow a choice to be made between 70% inhibition of the transfer step in all the molecules and 100% inhibition of this step in 70% of the molecules.

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