"TRANSFORMATION" OF MITOCHONDRIAL MONOAMINE OXIDASE INTO A DIAMINE OXIDASE - LIKE ENZYME IN VITRO

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Although soluble amine oxidases (diamine-, spermine-, and benzylamine-oxidases) differ considerably (Blaschko, 1963) in their substrate specificity and sensitivity towards the effect of inhibitors from structure-bound mitochondrial monoamine oxidases (MAO), similarity in mechanisms of deamination catalyzed by various amine oxidases appeared probable (Braunstein and Shemyakin, 1953; Braunstein, 1958).

In continuation of our previous experiments on the problem of "multiplicity" of MAO (Gorkin, 1963; Gorkin, 1966) we have found (Rapava et al.,1966) that peroxides of higher unsaturated fatty acids (e.g. oleic) completely inactivate MAO in course of preincubation; at the same time content of SH groups in the enzyme preparation decreases. Inhibition of the enzymatic activity could be prevented by dialysis of samples immediately after addition of peroxidized oleic acid.

If the reactions of deamination of tyramine and serotonin are catalyzed by different enzymes (or active sites), one may expect that in presence of a saturating concentration of one of the substrates (e.g. serotonin) the peroxidized oleic acid will inhibit in course of preincubation mostly the deamination of another substrate (e.g. tyramine). After the dialysis one might obtain an enzyme preparation possessing the ability to deaminate mainly serotonin.

Experimental. The hypothesis formulated above was partially confirmed in our experiments (Table 1). After treatment of rat liver mitochondrial MAO by peroxidized oleic

acid in presence of saturating concentration of serotonin with subsequent dialysis maximal specific rate of deamination of tyramine is decreased by about 90 per cent while that of serotonin - only by 34 per cent. The difference is statistically significant ($P<0.01$).

TABLE 1

Substrate specificity of "transformed" mitochondrial amine oxidase

Substrates and their final concentrations in samples	Liberation of ammonia nitrogen in mμmoles for 1 minute per 1 mg of protein by enzyme preparations:	
	initial	"transformed"
Tyramine·HCl (3.2×10^{-3} M)	16.0 ± 0.6 (6)	1.6 ± 0.3 (6)
Serotonin creatinine sulphate (5×10^{-3} M)	6.0 ± 0.7 (6)	4.0 ± 0.6 (6)
Tryptamine·HCl (1×10^{-3} M)	3.4 ± 0.1 (3)	1.5 ± 0.1 (3)
Dopamine·HCl (3×10^{-3} M)	3.7 ± 0.4 (3)*	2.3 ± 0.2 (3)
Histamine·2HCl (1×10^{-2} M)	0 (3)	2.3 ± 0.1 (6)
Spermine·4HCl (1×10^{-2} M)	0 (3)	2.1 ± 0.3 (6)
D,L-Lysine·HCl (1×10^{-2} M)	0 (3)	1.8 ± 0.2 (6)

Rat liver mitochondria were washed by 0.01 M phosphate buffer, pH 7.4 and lyophilized (Gorkin and Veryovkina, 1963). To a suspension of this preparation (10-14 mg/ml) in 0.004 M phosphate buffer, pH 7.4 were successively added serotonin creatinine sulphate (final concentration 5×10^{-3} M) and peroxidized (Rapava et al., 1966; table 2) oleic acid (final concentration 1×10^{-2} M). The samples were incubated for 2 hours at 37°C in oxygen and dialysed for 48 hours against 500-fold excess of 0.004 M phosphate buffer, pH 7.4 (three changes of buffer solution). Control samples were incubated and dialysed in identical conditions but without addition of peroxidized oleic acid. Rates of deamination were measured by liberation of ammonia for 45 minutes of incubation (during which the reaction follows zero order kinetics) in presence of saturating concentrations of the substrates. In experiments marked (*) the V_{max} values were calculated on the bases of K_m determined in preliminary experiments. Methods for estimation of ammonia and protein were as indicated before (Gorkin et al., 1964). Figures in the table are mean values ± S.E.M. (in parentheses - - number of experiments).

The enzyme preparation thus obtained retains also to a considerable degree the ability of an initial one to de-

aminate tryptamine or dopamine but the most striking new property aquired by the modified in this way ("transformed") amine oxidase is its ability to catalyze deamination of typical substrates of diamine oxidase from animal tissues (histamine) of soluble spermine oxidase (spermine), and of diamine oxidase from plant tissues (lysine). The last observation might be relevant to the problem of enzymatic deamination of lysine in process of elastine formation (Kim and Hill,1966).

Contrary to mitochondrial MAO the "transformed" enzyme prepared in conditions described above is resistant towards the inhibitory effect of the most potent MAO inhibitors (pargyline and tranylcypromine). At the same time three different carbonyl reagents distinctly inhibit the activity of "transformed" amine oxidase (Table II).

TABLE II

Sensitivity of the "transformed" mitochondrial amine oxidase towards the effect of some metabolic inhibitors.

Inhibitors	Inhibition of deamination (in per cent in relation to samples without the inhibitors) by the enzyme preparations:	
	initial	"transformed"
N-methyl-N-benzyl-propynylamine·HCl (pargyline)	100	0 (3)
trans-2-phenylcyclo-propylamine·1/2 H_2SO_4 (tranylcypromine)	100	0 (3)
Harmine·HCl	100	0 (3)
KCN	0	30 ± 8 (3)
Hydroxylamine·HCl	0	45 ± 5 (4)
Isonicotinoilhydrazide (isoniazid)	0	43 ± 4 (4)

Composition of samples and experimental conditions as in legend to the table 1. Inhibitors (final concentrations in samples in all the cases 1×10^{-3}M) were preincubated with the enzyme preparations as was described previously (Veryovkina et al.,1964; Gorkin et al.,1966). Substrate -- serotonin creatinine sulphate (5×10^{-3}M).

Discussion. On the basis of experiments (Weissbach et al.,1961) showing that even high doses of iproniazid or ß-phenylisopropylhydrazine administered in vivo do not cause complete inhibition of formation of 5-hydroxyindol-

eacetic acid or indoleacetic acid from serotonin or tryptamine, respectively, it was concluded (Weissbach et al., 1961) that some fraction (about 1-4 per cent of the total activity) of MAO in animal tissues is resistant towards the effect of MAO inhibitors.

After heating of rat liver homogenates for 10 minutes at 60°C a part of MAO activity measured by oxidation of serotonin was recovered (Nagatsu and Yagi,1965) but the sensitivity of the enzyme towards the inhibitory effect of a number of MAO inhibitors was lost.

The striking changes in substrate specificity and in sensitivity towards the effect of inhibitors of the rat liver mitochondrial MAO described above were caused by treatment of an enzyme preparation, in condition of protection of the active site of MAO by a substrate, by means of peroxides of a higher fatty acid which may form and accumulate in animal tissues in normal and pathological states (Gorkin, 1957). If the transformation of mitochondrial MAO into a diamine oxidase-like enzyme similar to that described by us actually occurrs in animal tissues, this process might possibly prove to be of interest for elucidation of enzymatic mechanisms of some biochemical reactions involving the deamination of aminogroups (deamination of polyamines, epsilon-aminogroup of lysin, gamma-aminobutiric and delta-aminolevulinic acid, ets.).

Studies on conditions of preparation and properties of the "transformed" mitochondrial amine oxidase are in progress.

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