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The release of tritium upon deamination of 3,4-dihydroxy[2-^{3}H]phenylethylamine by plasma amine oxidase

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SUMMARY

Deamination of dihydroxy[2-^{3}H]phenylethylamine (dopamine) by plasma amine oxidase (monoamine O_2 oxidoreductase (deaminating), EC 1 4 3 4) results in the release of one of the two tritium atoms in the 2 position of the molecule This phenomenon does not occur with mitochondrial amine oxidase

Amine oxidases can be divided into two types based on the presence or absence of the coenzyme pyridoxal in the protein molecule[1] Liver mitochondrial amine oxidase does not contain this coenzyme whereas both plasma amine oxidase (monoamine O_2 oxidoreductase (deaminating), EC 1 4 3 4) and histaminase (diamine O_2 oxidoreductase (deaminating), EC 1 4 3 6) do It has recently been observed by Beaven and Jacobsen[2] that the deamination of histamine labeled with tritium in the β position results in the complete release of tritium atoms, whereas similarly labeled [β-^{3}H]-methylhistamine is deaminated by mitochondrial amine oxidase with full retention of the tritium in the methyl imidazole acetaldehyde Beaven and Jacobsen[2] suggested that this finding was consistent with the mechanism for histamine deamination proposed by Werle and Pechmann[3] in which the release of tritium might be dependent on the ability of the coenzyme-substrate complex to form a hyperconjugated system during oxidation

It was of interest to examine whether the tritium release phenomenon observed with histaminase also occurred with this pyridoxal-containing plasma amine oxidase Using 3,4-dihydroxy[2-^{3}H]phenylethylamine ([2-^{3}H]dopamine) as substrate, the release of the β-tritium as water was measured during deamination by plasma and liver mitochondrial amine oxidase

Plasma amine oxidase was a gift from Dr Donald Reed of the Oregon State University, Corvallis, Oreg , U S A This enzyme had been partially purified from steer plasma and had a specific activity of 0 88 μmole benzylamine oxidized per mg protein per

h Guinea pig livers were used as source for the mitochondrial enzyme The livers were homogenized in 10 vol of 0 25 M sucrose and a crude mitochondrial fraction was prepared by differential centrifugation This material had a specific activity of 0 27 μmole benzylamine per mg protein per h

[2-^{14}C] Dopamine, [2-^{3}H] dopamine, and [2-^{3}H] phenylethylamine were purchased from New England Nuclear Corp [α-^{14}C] Benzylamine was purchased from Nuclear Research Chemicals Each of the radioactive amines was diluted with unlabeled amine to give a final specific activity of 10 μC/μmole β-Phenylisopropylhydrazine (JB-516) and pargyline hydrochloride★ (MO 911) were supplied by Lakeside Laboratories and Abbott Laboratories, respectively

Deamination of [^{14}C] dopamine and benzylamine were assayed by the IRC-50 column technique described previously by Robinson *et al* [4] in which the deaminated metabolites, dihydroxyphenyl acetaldehyde or benzaldehyde, remain in the column effluent The release of tritiated water from [2-^{3}H] dopamine was measured by distillation of a portion of the water from the incubation medium Radioactivity measurements were made with a Packard Tri-Carb liquid scintillation spectrometer and were corrected to disint /min by the external standardization technique The aqueous samples were counted in omnifluor scintillation solution (New England Nuclear Corp)

Deamination of [^{3}H] dopamine by plasma amine oxidase resulted in the release of approximately 50% of the tritium at the 2 position (Table I) Conversely mitochondrial amine oxidase did not appreciably labilize these protons during the deamination process

TABLE I

OXIDATIVE DEAMINATION OF DOPAMINE BY AMINE OXIDASES

The data are the average results of duplicate experiments using 10^{-4} M [2-^{14}C]- or [2-^{3}H] dopamine (1 μC), 0 1 M potassium phosphate buffer, pH 7 2, and 21 and 3 units of plasma and mitochondrial amine oxidase, respectively, in a volume of 1 ml 1 unit of enzyme activity is that amount which will oxidize 1 μmole of benzylamine per h The ^{14}C and ^{3}H experiments were done in separate assay tubes and the amount of the tritium released calculated on the assumption that each radioactive molecule contained 2 atoms of ^{3}H at the 2 position

Time of incubation (min)	*Plasma amine oxidase*		*Mitochondrial amine oxidase*	
	[^{14}C] DHPA formed (nmoles)	*^{3}H released (natoms)*	*[^{14}C] DHPA formed (nmoles)*	*^{3}H released (natoms)*
10	31 2	30 4	3 2	0 5
20	46 8	41 2	6 5	0 7
30	54 5	49 6	9 1	1 4

Abbreviation DHPA, dihydroxyphenyl acetaldehyde

The enzymic specificity of the tritium release was examined by using specific inhibitors of the two types of enzymes Pargyline is an effective inhibitor of the mitochondrial amine oxidase only, whereas JB-516 inhibits both plasma and

★Pargyline is *N*-methyl-*N*-benzyl-propynylamine

mitochondrial amine oxidase[4] KCN is an inhibitor of plasma amine oxidase As seen in Table II, inhibition of tritium release by plasma amine oxidase was very similar to the inhibition of dihydroxyphenyl aceteldehyde formation, suggesting that the tritium release was related directly to the enzymic deamination The same concentration of the inhibitors used in the above experiments showed the following degree of inhibition when the deamination was catalyzed by mitochondrial amine oxidase pargyline, 100%, JB-516, 98%, KCN, 52% Mitochondrial amine oxidase, however, catalyzed little or no release of the tritium atoms in the 2 position

TABLE II

INHIBITION OF PLASMA AMINE OXIDASE

Assays were done in duplicate using 21 units plasma amine oxidase and 10^{-4} M substrate at 37° for 15 min in a volume of 1 ml In this experiment [^{3}H]DHPA formation was measured by the IRC-50 column technique using [2-^{3}H]dopamine as a substrate

	Enzyme activity		*Inhibition (%) of*	
	^{3}H released (natoms)	*[^{3}H]DHPA formed (nmoles)*	*^{3}H released*	*^{3}H[DHPA] formed*
Control	16 1	13 5	–	–
Pargyline (10^{-5} M)	15 8	13 5	2	0
JB 516 (10^{-5} M)	0 2	0 3	98	98
KCN (10^{-3} M)	9 8	8 4	39	38

Abbreviation DHPA, dihydroxyphenyl acetaldehyde

Since small amounts of tritium appeared to be released during mitochondrial deamination of dopamine, it was essential to determine if this release was related to the enzyme activity It was found that this release was completely suppressed by pargyline in concentrations sufficient to completely inhibit oxidative deamination While this tritium release was related to the formation of dihydroxyphenyl acetaldehyde, it appeared to be due to a lability of the aldehyde product and not the result of an enzymic mechanism This was shown in an experiment in which a large amount of mitochondrial monoamine oxidase was used so that approximately 80% of the [2-^{3}H]dopamine was oxidized in 10 min The experiment was continued for another 10 min in which little additional dopamine was oxidized During the second 10-min period the release of tritium continued at a linear rate suggesting that this slow release of tritium may be due solely to lability of the tritium on dihydroxyphenyl acetaldehyde

The tritium release phenomenon has also been observed using [2-^{3}H]phenylethylamine In this case also only one of the two tritium atoms in the 2 position was released during oxidative deamination by plasma amine oxidase No release was observed during deamination of this substrate by mitochondrial amine oxidase

The substrate [2-^{3}H]dopamine used in these experiments was prepared by catalytic reduction of arterenone, thus having two tritium atoms in the 2 position The results suggest that only one of the two tritium atoms is labilized by oxidative deamination by plasma amine oxidase It would be important in terms of mechanism to know the stereochemistry of this tritium removal There appears, however, to be no convenient

procedure to prepare (+)- and (−)-[2-^{3}H]dopamine It would appear from these data that a mechanism similar to that proposed by Werle and Pechmann[3] for histaminase is also operative for the pyridoxal phosphate-containing plasma amine oxidase In this case the azomethine (coenzyme-substrate complex) would be oxidized and the intermediate formation of an unsaturated complex would be facilitated by the aromatic ring of the substrate Presumably the oxidation is catalyzed by the enzyme-bound copper in plasma amine oxidase[1] following Schiff base formation The loss of only one tritium, however, suggests that this mechanism may differ from that of histaminase The difference may lie in the stability or lability of the oxidized substrate-enzyme intermediate

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