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N-methylation of 1-methyltryptamines by indolethylamine *N*-methyltransferase

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Our finding that the methyltetrahydrofolate-mediated *N*-methyltransferase did not methylate the amino-nitrogen [1] led us to suggest a possible methylation on the indole ring nitrogen to give 1-methyl derivatives. As a part of our continuing studies on the kinetics of indolethylamine *N*-methyltransferase (INMT), we have used two 1-methyl derivatives of tryptamine, 1-methyltryptamine (1-MeT) and 1-methyl-*N*-methyltryptamine (1-MeNMT), as substrates in order to study the relationship between the structure of the substrate and the activity of the enzyme. The effects of *N*,*N*-dimethyltryptamine (DMT), bufotenin (Aldrich Chemical Co., Milwaukee, Wis.), 1-methyl-*N*,*N*-dimethyltryptamine (1-MeDMT) and *S*-adenosylhomocysteine (SAH) (Sigma Chemical Co., St. Louis, Mo.) on the activity of the enzyme were also examined. The results of our experiments are reported in this communication.

INMT was obtained from rabbit lung [2]. Fifty milligrams of lyophilized human serum was also used as a source of enzyme. The assay medium, which contained 0·5 m-mole *S*-adenosyl[methyl-^{14}C]methionine (SAM, Amersham–Searle Corp., Arlington Heights, Ill., sp. act. 51 mCi/m-mole), 0·5 μmole non-labeled SAM (Sigma Chemical Co., St. Louis, Mo.), 100 μg enzyme, amine [*N*-methylserotonin (NMS), *N*-methyltryptamine (NMT) (Aldrich Chemical Co., Milwaukee, Wis.), 1-MeT or 1-MeNMT] in various amounts and 50 μmoles potassium buffer (pH 8) in a total volume of 0·5 ml, was incubated at 37° for 60 min. The methylated products were extracted with ethyl acetate at pH 10, and the radioactivity was measured [3].

To identify the methylated products, two-dimensional t.l.c. of the ethyl acetate extract and of reference standards was carried out on Silica gel G [4]. The products were visualized by spraying the plates with methanolic sulfuric acid. Those fluorescent spots isographic with the standards were scraped, eluted with methanol and the radioactivity in the eluate was counted [3].

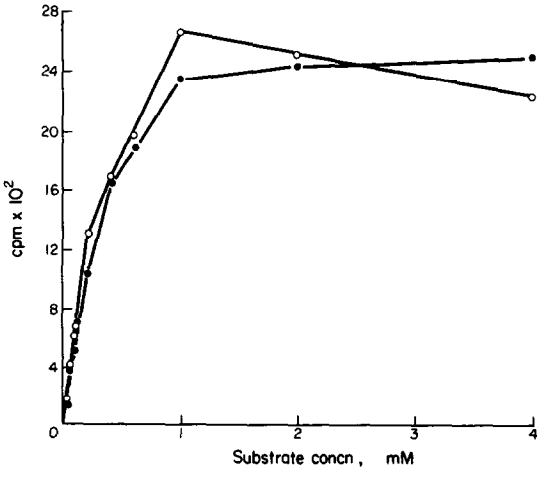

Fig. 1. Effect of substrate concentration on the *N*-methylation of 1-methyltryptamine (1-MeT, solid circles) and 1-methyl-*N*-methyltryptamine (1-MeNMT, open circles) by indolethylamine *N*-methyltransferase from rabbit lung.

G.l.c.–m.s. analyses and quantitation of the reaction products were carried out on a Varian CH 7 mass spectrometer interfaced with a Varian model 2740 gas chromatograph. The g.l.c. conditions were: 6-ft column, 3% OV-225 on Gas Chrom Q at 180° isothermal, helium flow-

Table 1. G.l.c. and g.l.c.–m.s. data of the methylation products of 1-methyltryptamine (1-MeT) and 1-methyl-*N*-methyltryptamine (1-MeNMT) with indolethylamine *N*-methyltransferase from rabbit lung

Compound	R_T* (min)	G.l.c.–m.s.†		
1-MeNMT				
Standard	6·66	188‡ (3)	144 (100)	145 (90)
Product of 1-MeT	6·66	188‡ (3)	144 (100)	145 (90)
1-MeDMT§				
Standard	5·53	202‡ (4)	144 (13)	58 (100)
Product of 1-MeNMT	5·53	202‡ (4)	144 (13)	58 (100)

* G.l.c. column conditions: 3% OV-225 on Gas Chrom Q, 6-ft column, 180° isothermal, He 30 ml/min.
† *m/e* (relative abundance).
‡ Molecular ion.
§ 1-Methyl-*N*,*N*-dimethyltryptamine.

Table 2. Kinetic parameters for indolethylamine *N*-methyltransferase from rabbit lung*

Substrate	K_m (M)	V_{max} (nmoles)
N-methylserotonin	2.5×10^{-4}	3·6
N-methyltryptamine	0.7×10^{-4}	3·5
1-Methyltryptamine	4.5×10^{-4}	3·5
1-Methyl-*N*-methyltryptamine	4.2×10^{-4}	4·0

* Assay conditions are described in the text.

Table 3. Inhibition of indolethylamine *N*-methyltransferase by 1-methyl-*N*,*N*-dimethyltryptamine (1-MeDMT), *N*,*N*-dimethyltryptamine (DMT), bufotenin and *S*-adenosylhomocysteine (SAH)*

Substrate (1×10^{-3} M)	Inhibition (%) produced by 1-MeDMT (1×10^{-3} M)	DMT (1×10^{-3} M)	Bufotenin (1×10^{-3} M)	SAH (1×10^{-4} M)
N-methylserotonin	83	90	85	81
N-methyltryptamine	60	83	75	83
1-Methyltryptamine	95	95	95	88
1-Methyl-*N*-methyltryptamine	83	88	80	82

* Assay conditions are described in the text.

rate of 30 ml/min. The spectra were obtained at an ionizing energy of 70 eV and separator and ion source temperature of 300°.

1-MeT and 1-MeNMT were methylated *in vitro* by INMT from both rabbit lung (Fig. 1) and human serum. The products of the methylation were identified as 1-MeNMT and 1-MeDMT, respectively, by t.l.c. and g.l.c.–m.s. Radioactivity was found in the spots isographic with reference standards. The methylated products were located by co-chromatography with unlabeled standards on t.l.c. 1-Methyltryptamines gave no color when sprayed with *p*-dimethylaminocinnamaldehyde and did not fluoresce when sprayed with *o*-phthalaldehyde, but fluoresced intensely under ultraviolet light when sprayed with methanolic sulfuric acid.

1-MeT, 1-MeNMT and 1-MeDMT were separated by g.l.c. The mass spectra have recently been reported [5]. The retention times and the mass spectra of 1-MeNMT and 1-MeDMT from the enzymatic methylation of 1-MeT and 1-MeNMT were identical with those of the reference standards (Table 1).

Quantitation of 1-MeNMT and 1-MeDMT by monitoring the ions at *m/e* 144 and 58, respectively, showed that both 1-MeT and 1-MeNMT are good substrates for the enzyme. Two samples of human serum incubated with 1-MeNMT yielded, respectively, 0·22 and 0·24 μg 1-MeDMT. Unlike aromatic-L-amino acid decarboxylase [6], the activity of INMT does not require free hydrogen on the ring nitrogen of indoleamines. The present experiments show that 1-methyltryptamines may undergo further changes with SAM-dependent INMT to 1-MeNMT and 1-MeDMT. The major product of 1-MeT is 1-MeNMT with a 10 per cent yield of the dimethylated tryptamine, 1-MeDMT.

At a high concentration (4×10^{-3} M) 1-MeNMT, and not 1-MeT, acted as a substrate inhibitor (Fig. 1). Similar substrate inhibition has also been reported for *N*-methyltryptamine [4, 7]. Reciprocal plots [8] revealed K_m values higher than those for NMS and NMT, whereas the V_{max} values were the same (Table 2). The rate of *N*-methylation of 1-MeT and 1-MeNMT was inhibited by the end products, 1-MeDMT, DMT and bufotenin, and by SAH (Table 3). Similar product inhibition of INMT has recently been demonstrated [2, 7, 9, 10].

The psychotogenic dimethylated tryptamines, bufotenin and DMT, have been reported to occur in urine of schizophrenic patients [11, 12]. In view of the established presence of methyltetrahydrofolate-dependent [13] and SAM-dependent *N*-methyltransferases [4], the physiological role of 1-methyltryptamine derivatives merits further investigation.

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