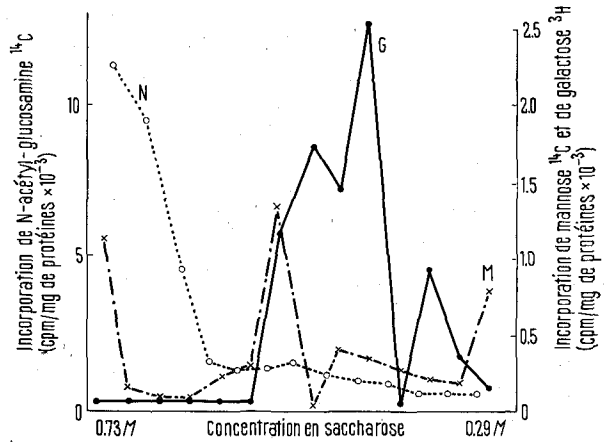
10 ml de saccharose 1,6 *M* et centrifugés pendant 30 min à 75000 *g*, une butée de 2 ml de saccharose 1 *M* servant d'intermédiaire entre les deux couches, permettant ainsi une meilleur récupération de la fraction dans une concentration de saccharose connue. Les membranes endoplasmiques, recueillies dans le saccharose 1 *M*, sont amenées à une concentration en saccharose 0,24 *M*. Cette dernière suspension est centrifugée pendant 75 min, à 20000 t/m, contre un gradient linéaire de saccharose 0,29 *M*–0,73 *M* (en tampon *Tris*-HCl 0,05 *M*, pH 7), dans le rotor SW 25–1 d'une ultracentrifugeuse Spinco. On recueille des fractions aliquotes, dont on apprécie l'activité glycosyl-transférase en système acellulaire in vitro. Pour chaque fraction étudiée, les systèmes acellulaires de biosynthèse comprennent: 400 µl de la suspension de membranes endoplasmiques contenant de 40 à 120 µg/ml de protéines. 10 µl de $MnCl_2$ (concentration finale 5.10^{-3} *M*). 10 µl d'une solution de précurseur radioactif contenant, selon l'activité glycosyl-transférase étudiée: soit 2,5 µCi d'UDP-galactose ^3H (NEN Corporation); soit 2 µCi de GDP-mannose ^{14}C (NEN Corporation); soit 1 µCi d'UDP-N-acétyl-glucosamine ^{14}C (NEN Corporation).

Les incubations sont réalisées à 30 °C pendant 30 min. Au terme du temps de biosynthèse, les macromolécules glycoprotéiniques sont précipitées sur filtres Watman et la radioactivité est évaluée en scintillation liquide, dans des conditions précédemment décrites[6,7].

La figure rend compte des résultats obtenus. Elle met en évidence une hétérogénéité de répartition des activités glycosyl-transférases, à partir des formes coenzymatiques actives des oses ou osamines, sur des accepteurs protéiniques endogènes: galactosyl-transférase, mannosyl-transférase et N-acétyl-glucosaminyl-transférase. On localise dans la fraction lourde des membranes endoplasmiques (fond de gradient) une double activité de transfert: mannosyl-transférase et N-acétyl-glucosaminyl-transférase. Dans la fraction de densité moyenne, on retrouve principalement les activités de transfert du mannose et du galactose. Dans la zone des structures membranaires légères, on retrouve également la mannosyl-transférase et la galactosyl-transférase, mais pas d'activité N-acétyl-glucosaminyl-transférase.

Il apparaît ainsi que les différents systèmes enzymatiques membranaires de transfert des oses ou dérivés d'oses occupent des zônes variables dans la structure des membranes endoplasmiques. Ceci pourrait éventuellement être utilisé comme un argument favorable à l'organisation séquentielle des enzymes dans les membranes proposée par Spiro[8] ou Schachter[9]. En fait, la méthode utilisée ne permet de séparer, sur le critère de densité, que des structures préférentielles de localisation. Ces résultats sont plutôt en faveur d'une dispersion de groupes de glycosyl-transférases dans l'ensemble du reticulum endoplasmique et de ce fait, peuvent s'intéger dans le cadre de la théorie «de l'espace vital» émise par l'un d'entre nous[4], selon laquelle l'organisation tridimensionnelle de la protéine conditionne à elle seule, grâce aux espaces disponibles qu'elle tolère entre les résidus amino-acyls, la fixation secondaire des éléments glucidiques, grâce à des systèmes enzymatiques à répartition ubiquitaire.



Répartition des activités glycosyl-transférases dans les membranes du reticulum endoplasmique, sur des accepteurs protéiniques endogenes à partir des formes coenzymatiques actives des oses ou osamine. M, mannosyl-transferase; G, galactosyl-transferase; N, N-acetyl-glucosaminyl-transferase. Gradient de saccharose 0,29 *M*-0,73 *M*.

Summary. In smooth endoplasmic membranes of rat splenic cells, glycosyl-transferase activities are located in various structures. These structures are separated by sucrose density gradient centrifugation. At the bottom are mannosyl- and N-acetyl-glucosaminyl-transferases; in the medium are mannosyl- and galactosyl-transferases; at the top are also mannosyl- and galactosyl-transferases, but no N-acetyl-glucosaminyl-transferase.

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Guaiacol-*O*-Methyltransferase: A Mammalian Enzyme Capable of Forming Di-*O*-methyl Catecholamine Derivatives[1]

We have reported previously that N-acetyl-3-hydroxy-4-methoxyphenethylamine (iso-N-acetylmethoxytyramine, referred to as i-NAMT in this report) can be converted to N-acetyl-3, 4-dimethoxyphenethylamine (NADMPEA) by mammalian tissues[2]. The methyl donor for this reaction was shown to be S-adenosyl-methionine. The product of the reaction, NADMPEA, is biologically active[3] and the substrate, i-NAMT, has been shown to be a dopamine metabolite[4]. In this report evidence is presented that this reaction is catalyzed by a previously undescribed methyltransferase which is active against various guaiacol derivatives, principally 3-hydroxy, 4-methoxy substituted

acidic and neutral metabolites of catecholamines. This enzyme will be referred to as guaiacol-*O*-methyltransferase (GOMT).

Details of the enzyme assay have been reported previously[2]. In the assay a 100,000 × *g* 1 h supernatant fraction of liver or brain from Wistar rats was prepared in pH 9.0 *tris*-HCl buffer, and carefully dialyzed. Protein concentration was determined by the method of LOWRY[5]. A typical assay for the first substrate tried, i-NAMT, contained 5 mg protein, 0.48 μmoles i-NAMT, 9.6 μmoles C^{14} S-adenosylmethionine (S.A. 52.5 mC/mmole), 10 μmoles $MgCl_2$. The reaction was carried out in 0.1 *M tris*-HCl buffer, pH 9.0 at 37 °C for 30 min in a final volume of 1 ml. The product was recovered from the incubation mixture by $CHCl_3$ extraction at pH 2. The concentrated extract was mixed with 200 μg authentic, unlabelled NADMPEA, applied to silica gel on fiberglass plates (Gelman Inst. Co.) and developed first in benzene-heptane-diethylamine, 10:5:1, and then in toluene-methanol-water, 750:35:1. The area of the chromatogram corresponding to NADMPEA was cut out. Radioactivity associated with the product was determined in a liquid scintillation counter. The identity of the product was proved by chromatography in various solvent systems, co-crystallization to constant specific activity with authentic NADMPEA and by the preparation and identification of 3 derivatives, each of which, after recrystallization, retained radioactivity associated originally with NADMPEA[2]. The assay was modified for other substrates used subsequently (see Table III).

Results of incubations of i-NAMT with soluble liver fraction are reported in Table I. The K_m against i-NAMT is $4.8 \times 10^{-4} M$. The enzyme is strongly Mg^{++} dependent, maximal activity having been found with $10^{-1} M$ Mg^{++} (Table II). The pH optimum is approximately 9. In addition to the studies with C^{14}-methyl-SAM and unlabelled i-NAMT reported in Table I, studies have been carried out with (C^{14} acetyl) i-NAMT and unlabelled SAM. Results of both approaches have yielded identical results.

[1] This work was supported by Public Health Service grants Nos. MH-08618 and MH-07961 and a Research Scientist's Award No. MH-14020, to A.J.F.

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Table I. Rate of conversion of i-NAMT to NADMPEA by rat liver

Experiment	Sample No.	Incubation system	Concentration i-NAMT (*M*)	nmoles NADMPEA formed/h/100 mg protein
A	1	Complete but denatured enzyme	1.5×10^{-3}	0.008
	2	Complete	1.5×10^{-3}	9.9
B	1	Minus substrate	0	0.006
	2	Complete	0.1×10^{-4}	0.150
	3	Complete	0.5×10^{-4}	0.766
	4	Complete	1.0×10^{-4}	1.44
	5	Complete	2.5×10^{-4}	3.02
	6	Complete	5.0×10^{-4}	5.59
	7	Complete	1.5×10^{-3}	10.00

Table II. Comparison of GOMT, COMT, HIOMT, POMT and IPOMT

Experimental condition	Relative activity GOMT	COMT	HIOMT	POMT	IPOMT
pH 6.0–6.8	<0.001	—	—	—	100
pH 7.9	1.1	100	0	100	—
pH 9.0	100	6.3	0	—	—
Mg^{++} (1.0 *M*)	26	10.9	—	—	—
($10^{-1} M$)	100	86.3	—	—	—
($10^{-2} M$)	88	100.0	—	—	—
($10^{-3} M$)	65	63.8	—	—	—
($10^{-4} M$)	28	12.7	—	—	—
($10^{-5} M$)	14		—	—	—
0	19	6.8	—	100	100
EDTA ($10^{-3} M$)	0	1.3	—	100	100
EDTA ($10^{-3} M$) plus Mg^{++} ($10^{-1} M$)	104	93.9	—	—	—

Substrates used were: i-NAMT $4.8 \times 10^{-4} M$ for GOMT, N-acetyl-dopamine $4.8 \times 10^{-4} M$ for COMT, N-acetylserotonin $4.8 \times 10^{-4} M$ for HIOMT. Methyl donor in each case was SAM. Data for POMT are from AXELROD and DALY[8] and for IPOMT from TOMITA et al.[9]. Other data are from experiments carried out in our laboratory. pH studies were carried out with 100,000 g × 1 h soluble fraction of liver. Mg^{++} studies were carried out with 30–50% saturated $(NH_4)_2SO_4$ precipitate of the soluble fraction. Precipitate was dialyzed prior to incubation and reaction was carried out at pH 9.0 for GOMT, and 7.9 for COMT. Relative activity refers only to comparisons within a column.

Table III. *O*-Methylation of various compounds by GOMT

Substrate	Relative activity	Expected product
iso-Homovanillic acid[a]	100	Dimethoxyphenylacetic acid
Homovanillic acid	9	Dimethoxyphenylacetic acid
iso-N-acetylmethoxytyramine[b]	45	NADMPEA
N-acetylmethoxytyramine[b]	11	NADMPEA
iso-Vanillylalcohol	23	Dimethoxybenzyl alcohol
Vanillyl alcohol	6	Dimethoxybenzyl alcohol
iso-Methoxytyramine	5	DMPEA
Methoxytyramine	3	DMPEA
iso-Vanillin	1	Dimethoxybenzaldehyde
Vanillin	2	Dimethoxybenzaldehyde
3-Methoxy-4-hydroxyphenethanol	11	Dimethoxyphenethanol
p-Tyramine	1	*p*-Methoxyphenethylamine
p-Hydroxyamphetamine	1	*p*-Methoxyamphetamine
Normetanephrine	0.5	Dimethoxyphenethanolamine
N-acetylserotonin	0	Melatonin

Incubates: 8 mg protein (100,000 $\times g$ 1 h dialyzed rat liver supernatant) 0.5 μmole substrate, 5 μmole $MgCl_2$, 0.2 μC C^{14}-methyl SAM, S.A. 52.5 mC/mmole and 20 μmole pH 9.0 *tris*-HCl buffer, final volume 0.5 ml. Samples incubated for ½ h at 37 °C. Acidic and neutral products extracted at pH 2, basic products at pH 13 with 90:10 toluene-isoamyl alcohol. Portion evaporated, chromatographed and C^{14} corresponding to product counted in liquid scintillation counter. In addition, after chromatography, the methylated product of the 3 substrates marked [a] or [b] were crystallized to constant specific activity with appropriate carrier, 3,4-dimethoxyphenylacetic acid[a] or NADMPEA[b]. For other proofs of identity of product see text.

Other enzymes which could conceivably catalyze this reaction are catechol-*O*-methyltransferase (COMT)[6], hydroxyindole-*O*-methyltransferase (HIOMT)[7], phenol-*O*-methyltransferase (POMT)[8] and iodophenol-*O*-methyltransferase (IPOMT)[9]. COMT has been shown to require an adjacent free hydroxyl group in order for *O*-methylation to take place. This requirement is not met by i-NAMT or other substrates for GOMT. HIOMT has been shown to be present almost exclusively in pineal and is not active against phenethylamine derivatives. The activity of POMT is restricted to the particulate fraction while 95% of the activity of GOMT is found in the soluble fraction, as is most of the COMT and IPOMT. The five enzymes are further compared in Table II. From these data it can be seen that GOMT is about 90 times more active at pH 9.0 than it is at 7.9, while COMT is about 15 times more active at pH 7.9 than it is at 9.0. No activity of HIOMT could be demonstrated in soluble liver preparations using N-acetylserotonin as substrate (Table II). Both GOMT and COMT have a high requirement for Mg^{++} and are completely inhibited by EDTA. POMT, on the other hand, is inhibited by Mg^{++}. Both POMT and IPOMT have no known metal ion requirement and are unaffected by EDTA.

The Table III is a summary of data obtained with a series of relevant substrates. All active substrates studied contained a guaiacol group. As can be seen, several of the best substrates for this enzyme are mono-*O*-methylated neutral and acidic metabolites of catecholamines. Further methylation of these substrates by GOMT results in the formation of di-*O*-methyl catecholamine derivatives.

Final proof that these dimethoxy compounds are formed via the action of a previously undescribed enzyme awaits purification of GOMT away from other methylating enzymes. However, the difference in substrate specificities and other characteristics constitutes strong presumptive evidence that GOMT is a different enzyme from the others discussed.

It is not known at present whether the action of this enzyme has physiological significance. However, the demonstration that mammalian tissues can form dimethoxy compounds is of obvious interest in the study of psychotic disorders since at least some of these compounds have mescaline-like effects[3].

Résumé. On a extrait et characterisé une enzyme capable de transférer aux catécholamines 4-*O*-méthyles le group méthyle provenant de l'S-adénosylméthionine et produire des substances diméthoxyques. Cette enzyme est active dans différents tissus de Mammifères.

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