Hydroxyindole-*O*-methyltransferase I: Substrate Binding

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Abstract □ The mode of binding of the substrate *N*-acetylserotonin on hydroxyindole-*O*-methyltransferase was studied. The indole nucleus, the amide C=O, and possibly the methylene group(s) have been found to be involved in the binding of *N*-acetylserotonin, whereas the 5-OH and the amide NH did not seem to contribute to the binding. There was a good indication that the CH_3 of the amide was probably bound to a hydrophobic region on the enzyme.

Keyphrases □ Enzyme inhibitors—synthesis, action mechanism □ Hydroxyindole-*O*-methyltransferase—substrate binding □ *N*-Acetylserotonin—hydroxyindole-*O*-methyltransferase binding □ IR spectrophotometry—structure, identity

Axelrod and Weissbach (1) in 1961 discovered an enzyme in pineal gland capable of methylating 5-hydroxy groups of indole derivatives. Since this enzyme is different from catechol-*O*-methyltransferase and is specific for hydroxyindoles, it was named hydroxyindole-*O*-methyltransferase (HIOMT) (2). Indole derivatives with hydroxyl group substituted on the 4, 5, or 6 position have been tested for substrate specificity (1). Of all the compounds tested, *N*-acetylserotonin was the best substrate for HIOMT, being *O*-methylated to 5-methoxy-*N*-acetyltryptamine (melatonin). Melatonin has been demonstrated to control the melanin granules in frogs (3) and exert certain effects on estrus and ovarian weight (4, 5). Its total physiological function in mammals, however, is not yet clearly understood. In view of the diversified implication of melatonin, a malfunction in the biosynthesis or metabolism of this neurohormone could possibly manifest itself in behavioral abnormalities. It is anticipated that if the HIOMT-catalyzed methylation of *N*-acetylserotonin could be blocked, the absence of melatonin could be evaluated and ultimately its functional role in the body defined.

Before a good inhibitor could be found, it is essential to know the mode of binding of the substrate to the enzyme. Information thus obtained can then be applied to design a more potent inhibitor. An effective way to study the substrate binding is to determine the relative affinities of inhibitors that are structurally similar to the substrate and to evaluate the effects of different substituents on the binding to the enzyme (6). This paper reports the result of the author's findings on the binding of *N*-acetylserotonin on HIOMT.

DISCUSSION

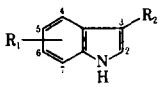
A total of six possible positions on the *N*-acetylserotonin molecule could account for the binding of the substrate to the enzyme, namely the oxygen of the 5-OH group, the indole nucleus, the two methylene groups on the C-3 chain, the hydrogen of the amide, the oxygen of C=O, and possibly the terminal CH_3 group. In general, any group that is involved in an enzymatic transformation will not participate in binding (7), therefore, the hydrogen of the 5-OH group will not contribute to the binding of *N*-acetylserotonin. The amide nitrogen does not offer any binding force, since the adjacent C=O group withdraws electrons and makes hydrogen bonding improbable.

The similarity in inhibitory activities (Table I) between II and III indicated that the methoxy group on C-6 position was most likely not bound to the enzyme. The same statement could also be applied to 5-methoxy group of I. The decrease in inhibitory activity of III by the substitution of the 5-methoxy group was probably due to the steric hindrance caused by the CH_3 of OCH_3.

Compound IV was synthesized for the evaluation of possible formation of hydrogen bonding between the hydrogen of –CONH– and the enzyme. Replacement of the NH by O, however, did not change the inhibitory activity of III, indicating that the NH was not involved in the binding. The binding of carbonyl groups in III and IV was obvious when their inhibitory activities were compared with that of VII. The absence of C=O caused a loss of 10-fold in activity.

There was good possibility that the terminal CH_3 of the amide (III) was bound hydrophobically to the enzyme, in view of the lower activity of V. At pH 7.9 most of V exists as an anion COO^-, and the repulsion between this charged species and the hydrophobic region on the enzyme would be anticipated. Further investigations of this hydrophobic bonding have been carried out and the results have been reported in the second paper of this series (8).

Indole (VIII) was found to be a weak inhibitor of the enzyme. It was apparently bound to the enzyme by the formation of a charge-transfer complex (9). When a methyl (IX), and ethyl (X), or a propyl (XI) group was placed in Position 3 of VIII, an increase in inhibitory activity of four-, seven-, and tenfold was observed, respectively. This result indicated the participation of the alkyl chain in hydrophobic bonding with the enzyme. It was a surprise to find that 3-propylindole (XI) was just as active as *N*-acetyltryptamine (III). At first glance it seemed as if the CH_3CO of III was not involved in the binding of III to the enzyme. A comparison of the activities between XI and VII, however, showed that this was not the case. It was not at all impossible that the terminal OH of VII, being a hydrophilic group, projected into a hydrophobic region on the enzyme whereon the terminal methyl group of XI was bound. This

Table I—Inhibition of HIOMT by 

Compd.	R_1	R_2	I_{50}[a] mM	K_i,[b] mM
I[c]	5-OCH_3	$CH_2CH_2NHCOCH_3$	2.80	0.53
II[d]	6-OCH_3	$CH_2CH_2NHCOCH_3$	1.65	0.31
III	H	$CH_2CH_2NHCOCH_3$	1.40	0.27
IV[e]	H	$CH_2CH_2OCOCH_3$	1.42	0.27
V[c]	H	$CH_2CH_2CH_2COOH$	14.9	2.8
VI[c]	H	$CH_2CH_2NH_2$	10.3	2.0
VII[c]	H	CH_2CH_2OH	13.2	2.5
VIII[c]	H	H	14.2	2.7
IX[c]	H	CH_3	3.4	0.65
X	H	CH_2CH_3	2.0	0.38
XI[c]	H	$CH_2CH_2CH_3$	1.5	0.29
XII	*N*-Acetylphenethylamine		12.0	2.3

[a] Concentration of an inhibitor giving 50% inhibition of the enzyme. [b] Calculated from $K_i = K_m \times [I]_{50}/[S]$, where $K_m = 5.7 \times 10^{-6} M$ and $[S] = 3 \times 10^{-4} M$. It is valid since $[S] > 5K_m(10)$. [c] Commercial sample. [d] This gift from the Research Department, Sandoz, Ltd., Switzerland, was greatly appreciated. [e] An oil.

could then cause a repulsion between the side chain of VII and the hydrophobic region on the enzyme and result in a loss of binding. Compound VII, having an inhibitory activity ninefold less than XI, was in fact, not any better as an inhibitor than indole (VIII). Even though a similar kind of repulsion could also be expected from the side chain NH of III and the hydrophobic region of the enzyme, the binding of C=O and CH_3 of III was able to compensate for the loss of activity.

N-Acetylphenethylamine (XII) showed weak inhibitory activity on HIOMT. It was noted that this compound bears an *N*-acetylaminoethyl side chain as well as a phenyl group which is capable of forming a charge-transfer complex with the enzyme. At the present moment no further information on the mode of binding of XII is available.

EXPERIMENTAL

Melting points are corrected and were taken on a melting-point apparatus.[1] IR spectra were obtained with a spectrophotometer.[2]

N-Acetyltryptamine (III)—A 5% aqueous NaOH solution was slowly added to a solution of 1 g. (5 mmoles) of tryptamine hydrochloride until the free amine just began to precipitate. Acetic anhydride (5 ml.) was added and the mixture was vigorously shaken. After addition of 25 ml. of 20% (w/v) aqueous sodium acetate, the resulting mixture was allowed to stand overnight at room temperature. The product was extracted with ether (3 × 50 ml.). The combined ethereal extracts were dried with anhydrous sodium sulfate, then evaporated under reduced pressure. The viscous residue was boiled with cyclohexane, and upon cooling an oil separated which solidified on standing to yield 0.22 g. (22%) of product, m.p. 73–74°.

A m.p. of 75–76° (11) has been recorded for this compound prepared by acetylation of tryptamine in the presence of potassium carbonate.

3-(2-Acetoxyethyl)indole (IV)—A solution of 0.8 g. (6.2 mmoles) of tryptophol in 10 ml. of acetic anhydride was refluxed for 6 hr., then evaporated under reduced pressure leaving a yellow liquid. $\lambda_{max.}$ (film) 2.93 (indole NH), 5.78–5.82 (C=O), 8.0 (C—O), and 13.45 μ (indole CH).

A hot solution of the above oil in 2 ml. of chloroform was mixed with a hot solution of 1.2 g. of picric acid in 18 ml. of chloroform. The resulting mixture was concentrated *in vacuo* to one-third of its volume, and red crystals deposited upon cooling inside a freezer. This monopicrate salt was collected on a filter and washed with 95% ethanol; yield, 0.9 g. (33.7%), mp. 100–100.5°. One recrystallization from chloroform gave cherry-red crystals, with the melting point remaining unchanged.

Anal.—Calcd. for $C_{18}H_{16}N_4O_9$: C, 50.0; H, 3.73; N, 13.0. Found: C, 50.1; H, 3.83; N, 13.0.

3-Ethylindole (X)—3-Acetylindole in tetrahydrofuran was reduced with lithium aluminum hydride to yield 4.6 g. (79%) 3-ethylindole, b.p. 77–78° (0.1 mm. Hg). The product solidified partially during the distillation, m.p. 31–34° [lit. (12) m.p. 42°]. Its IR spectrum showed the absence of an absorption peak at 6.2 μ due to C=O.

3-Propylindole (XI)—3-Propionylindole was obtained from the Grignard reaction (13). $LiAlH_4$ reduction of 3-propionylindole in tetrahydrofuran gave 5.7 g. (77%) of 3-propylindole, b.p. 74–76° (0.025 mm. Hg). The preparation of XI from 3-allylindole has been reported (14). The IR spectrum, which was identical to that reported (14), showed the absence of the absorption peak at 6.15 μ due to the C=O of 3-propionylindole.

N-Acetylphenethylamine (XII)—Phenethylamine was acetylated in a similar manner as in the preparation of III. Compound XII was obtained in a 78% yield, m.p. 52–53° [lit. (15) m.p. 45°]. $\lambda_{max.}$ (KBr) 3.05 (NH), and 6.10 μ (C=O).

Assay—Hydroxyindole-*O*-methyltransferase (75 mμmole/hr./mg. protein) was isolated from beef pineal gland and purified according to the method of Axelrod and Weissbach (1).

The stock solutions of Inhibitors IV–VIII were prepared in propylene glycol. Compounds II and III were dissolved in 50% aqueous propylene glycol, I and XII in 20% aqueous propylene glycol, and IX–XI in dimethylsulfoxide. The same magnitude of inhibitory activity was obtained regardless of the use of the above two solvents. For instance, the I_{50} of III in 50% dimethylsulfoxide was 1.5 m*M* and a value of 1.4 (Table I) was found when III was in an equal amount of 50% propylene glycol.

Incubation was carried out at 37° for 30 min. in a solution containing 0.3 mμmole of *N*-acetylserotonin, 0.3 mμmole of *S*-adenosylmethionine-^{14}C, varying amounts of inhibitor in equal amounts of organic solvent, 80 μl. of enzyme, phosphate buffer 7.9, and water to make a final volume of 1 ml. The control tubes contained the same amount of organic solvent, but without an inhibitor. The product was extracted and washed according to the procedure reported in *Reference 1*, then assayed for ^{14}C in a liquid scintillation spectrometer and the concentration of inhibitor at which the enzyme activity was 50% inhibited was determined.

[1] Fisher-Johns.
[2] Perkin-Elmer, model 237B.

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