SUBSTRATE SPECIFICITY AND HETEROGENEITY OF N-METHYLTRANSFERASES

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SUMMARY:

Histamine N-methyltransferase (EC. 2.1.1.8) from cat intestine and guinea pig brain was compared with indoleamine N-methyltransferase from rabbit lung and chick brain. Histamine N-methyltransferase, regardless of its source, methylated specifically histamine and not other imidazoles or aromatic amines. In contrast, indoleamine N-methyltransferase from rabbit lung had different substrate specificity from that of chick brain enzyme. The best substrate for lung indoleamine N-methyltransferase was N-methyltryptamine whereas serotonin was the best substrate for the chick brain enzyme. Both histamine N-methyltransferase and indoleamine N-methyltransferase appeared to occur in these species in multiple forms. They had different kinetic parameters, different pH optimum values, displayed different response towards inhibitors, and had different heat stability.

INTRODUCTION:

There are several methyltransferases in nature that are involved in the activation and inactivation of biogenic amines. These enzymes, all of which require S-adenosylmethionine as the methyl donor, include phenylethanolamine N-methyltransferase, catechol O-methyltransferase, histamine N-methyltransferase, hydroxyindole O-methyltransferase, and indoleamine N-methyltransferase (1-6). However, they may occur in tissues in multiple forms. It has been reported that adrenal phenylethanolamine N-methyltransferase and pineal hydroxyindole O-methyltransferase are heterogeneous among different species but are homogeneous within a given species. Histamine N-methyltransferase and catechol O-methyltransferase occur in multiple forms within a given

species as well as among different species (7). Furthermore, the substrate specificity of each of these enzymes from different sources may be different. Morgan and Mandell (6,8), for example, found that indoleamine N-methyltransferase from brains of chick, human and sheep methylates serotonin best in comparison to other amine substrates. In contrast, Mandel et al. (9) reported that rabbit lung indoleamine N-methyltransferase methylates N-methyltryptamine better than serotonin.

This paper compares histamine N-methyltransferase (HMT), which catalyzes the ring N-methylation of histamine to form methylhistamine, and indoleamine N-methyltransferase (IMT), which catalyzes the side chain N-methylation of aromatic amines, with respect to their substrate specificity. It also presents indirect evidence for the existence of multiple forms of these two enzymes in different species.

EXPERIMENTAL:

1. Preparation of Enzymes. Cat intestine and guinea brain were used as the enzyme source for HMT. Cats, 1.5-2.0 kg, or guinea pigs, 0.7-1.0 kg, were anesthesized with nembutal sodium given intraperitoneally (30-35 mg/kg) and then sacrificed by heart puncture. Cat intestines or guinea pig brains were removed rapidly and blotted dry on filter papers. The intestinal mucosa was homogenized in 8 volumes of ice-cold 0.25 M sucrose while the guinea pig brains were homogenized in 4 volumes of the same solute. The homogenate was centrifuged at 37,000 x g in a refrigerated Sorvall centrifuge for 1 hour. The supernatant was collected and recentrifuged at 144,000 x g for another hour in a spinco refrigerated centrifuge Model L2-65B. The supernatant was collected and used as the source of the enzyme HMT. It contained approximately 6.0-8.2 mg of protein per ml. When stored in a freezer at -15° it was stable for more than one week. It was also found that dialysis did neither affect the stability of the enzyme nor modify its activity or response towards heat and inhibitors.

For IMT, rabbits were sacrificed in the same manner as described above, and their lungs were removed rapidly. The lungs were then washed in isotonic saline and blotted dry on filter paper. For further steps in the preparation of rabbit lung IMT, the procedure of Axelrod (10) was followed up to the 40-60% ammonium sulfate fraction which was then dialyzed against four changes of large volume of 0.20 M potassium phosphate buffer, pH 7.9, for four hours. The dialyzed preparation containing about 12 mg protein per ml of the phosphate buffer was used as the enzyme source. For the preparation of chick brain IMT, white Leghorn cockerels (about one week old) were decapitated and the whole brain was dissected free from the pituitary stalk and pineal. The remaining procedure was identical to that described above for rabbit lung IMT. Since it was found that the activity of enzyme IMT prepared from both sources under these conditions was not stable and more than 50% of its activity was lost within 10 hours, the enzyme was used immediately after the preparation.

2. Assays for Enzymes. HMT activity was measured by the method described earlier (11,12). IMT was measured by the method of Mandel et al (9). Phenylethanolamine N-methyltransferase was assayed by the method of Axelrod, using norepinephrine or metanephrine as substrate (2). Catechol O-methyltransferase was measured by the method described in detail by Black (13). Protein was estimated by the method of Lowry et al. (14). Crystalline bovine serum albumin was used as the protein standard.

RESULTS:

A variety of substrates had been tested for their affinity for HMT from cat intestine and from quinea pig brain. These substrates include betazole, whose chemical structure closely resembles histamine, N-methyltryptamine, norepinephrine, serotonin and tryptamine. It was found that none of these substrates could serve as a substrate for the enzyme -- i.e., HMT methylated only histamine (Table 1). Furthermore, it was also found that the enzyme from other sources such as rabbit intestine and cat kidney had affinity

TABLE 1

SOME CHARACTERISTICS OF CAT INTESTINE AND GUINEA PIG BRAIN HISTAMINE N-METHYLTRANSFERASES (HMT)

Variable	Cat intestine HMT	Guinea pig brain HMT
Substrate	histamine only	histamine only
K_m (HIST)[a]	3.6×10^{-5}M	4.1×10^{-5}M
K_m (SAM)[a]	2.6×10^{-5}M	2.1×10^{-5}M
pH optimum	8.0 - 8.2	8.4
Heat stability: 55° for 4 min, % enzyme activity remaining[b]	70	54
Activation energy (Kcal/mol)[c]	13.7	18.6

[a] The K_m values for each substrate, histamine (HIST) or S-adenosylmethionine (SAM), were determined by the method of Lineweaver and Burk plots (16). For K_m (SAM), histamine concentration was fixed at 90 μM and SAM concentrations were varied between 20 and 150 μM. For K_m (HIST), SAM concentration was fixed at 100 μM and histamine concentrations were varied between 18 and 135 μM.

[b] Partially purified HMT (7.0 mg/ml) was heated at 55° at different time intervals. The residual enzymatic activity was determined as described in Experimental.

[c] Activation energy (E_a) was determined from the Arrhenius plot of log Vmax versus 1/T.

only for histamine and not other aromatic amines mentioned obove. However, HMT from cat intestine had different physicochemical properties from that of guinea pig brain. They had different Km values, different pH optimum values, different activation energy and different heat stabilities (Table 1). In addition, it was found that these two forms of the enzyme responded differently

towards different types of inhibitors. The inhibitors used were antimalarials, antihistaminics, and local anesthetics. In agreement with the findings of Cohn (15), the antimalarials are the most potent inhibitors of HMT known so far.

In contrast, indoleamine N-methyltransferase from rabbit lung and from chick brain had different substrate specificities (Table 2). The best substrate for rabbit lung IMT was N-methyltryptamine whereas serotonin was the best substrate for chick brain IMT. This is in agreement with the findings previously reported by Mandel et al. (9) and by Morgan and Mandell (6). Thus,

TABLE 2

SUBSTRATE SPECIFICITY OF INDOLEAMINE N-METHYLTRANSFERASES (IMT) FROM RABBIT LUNG AND CHICK BRAIN

Substrate	% IMT Activity[a]	
	Rabbit lung IMT	Chick brain IMT
Histamine	0	10
Norepinephrine	0	0
N-methyltryptamine	100	50
Serotonin	60	100
Tryptamine	80	60

[a] Partially purified enzyme (3.0 mg) from each source was tested for its affinity for each substrate as described in Experimental, N-methyltryptamine or serotonin being used as reference substrate respectively (that is, equal to 100%).

the present results suggest that multiple forms of IMT also occur in tissues of different species. This conclusion was further supported by the finding that IMT from the two sources had different kinetic parameters, yielded different percent inhibition by N,N-dimethyltryptamine, and had different sensitivity to heat (Table 3).

TABLE 3

CHARACTERISTICS OF RABBIT LUNG AND CHICK BRAIN INDOLEAMINE N-METHYLTRANSFERASES (IMT)

Variable	Rabbit lung IMT	Chick brain IMT
K_m (5-HT)[a]	1.0×10^{-3} M	7.8×10^{-4} M
K_m (SAM)[a]	0.8×10^{-4} M	1.6×10^{-5} M
% Inhibition by 10^{-4} M dimethyltryptamine[b]	90	100
Heat stability: 55° for 5 min, % enzyme activity remaining	60	40

[a] The K_m values for each substrate, serotonin (5-HT) or S-adenosylmethionine (SAM), were determined by the method of Lineweaver and Burk plots (16). For K_m (5-HT), SAM concentration was fixed at 24 μM and serotonin concentration varied between 50 and 400 μM. For K_m (SAM), serotonin concentration was fixed at 400 μM while SAM concentrations were varied between 5 to 30 μM.

[b] IMT activity was done as indicated in Methods at N-methyltryptamine or serotonin concentration of 4.0×10^{-4} M, the former substrate for rabbit lung enzyme whereas the latter for the enzyme from chick brain. Percent inhibition was estimated by comparing enzyme activity in the presence of dimethyltryptamine to that in the absence of the inhibitor. Each value represents the average of three determinations.

DISCUSSION:

Both HMT and IMT are enzymes involved in the N-methylation of aromatic imidazole derivatives, the chief difference between them being substrate specificity. The former enzyme, regardless of source, methylates histamine only, whereas the latter enzyme can methylate a variety of closely related amines. HMT occurs in multiple forms (isozymes) in tissues of a given species as well as among tissues of different species (7). The results presented herein also indicate that indoleamine N-methyltransferases from rabbit lung and chick brain are isozymes.

Evidence for the existence of biochemically distinguishable enzyme forms or isozymes comes from measurements of their physicochemical properties. Histamine N-methyltransferases from cat intestine and guinea pig brain had different kinetic parameters, different activation energy, displayed different behavior towards inhibitors, and had different heat stabilities. These results indicate strongly that the properties of cat intestine and guinea pig brain enzymes are not the same. Preliminary results also indicated that the two enzymes behave differently on DEAE-cellulose column.

There is little doubt from the results presented that indoleamine N-methyltransferases from rabbit lung and chick brain are isozymes since they have different substrate specificities and can be distinguished by differences in their biochemical properties. Recently, it has been reported that the enzymes from sheep and human brains have the same substrate specificity which is also identical to that of chick brain enzyme, i.e., serotonin is the best substrate for the three enzymes (8).

The functional significance of IMT is unknown at present, though it is well documented that HMT is one of the major enzymes involved in the inactivation of histamine outside the central nervous system. It is of particular interest to note the presence of these two enzymes in multiple forms in brains of some species including man (4,8,9). This implies that one form of the enzyme in one tissue may function differently from that in another tissue.

For example, methylhistamine formed in the brain by HMT may not be inactive. It could well be involved in the development of mental aberrations, as pointed out by Green (17). In addition, methylhistamine has been reported to be excreted in larger quantities in schizophrenics (18).

IMT catalyzes the formation of bufotenine and N,N-dimethyltryptamine; the latter compound is a potent psychotogenic agent in man (19). It has been identified in urine of schizophrenics (20,21,22), suggesting that the agent plays a role in psychoses. It would be interesting to see if both methylhistamine and N,N-dimethylhistamine are involved in the development of psychiatric disturbances. If so, the activities of these two methylating enzymes would be expected to increase in psychotic patients. This is, of course, a question awaiting future research.

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