The Specificity of Tryptophan Analogues as Inducers, Substrates, Inhibitors, and Stabilizers of Liver Tryptophan Pyrrolase*

MORTON CIVEN† AND W. EUGENE KNOX

From the Department of Biological Chemistry, Harvard Medical School, and the New England Deaconess Hospital, Boston, Massachusetts

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In animals, liver tryptophan pyrrolase was induced independently by administration of its substrate L-tryptophan (1), and of the hormone hydrocortisone (2). Some analogues of tryptophan which are not substrates of the tryptophan pyrrolase, such as α-methyl-DL-tryptophan (3) and β-tryptophan (3), have been reported to induce the enzyme in intact animals. Since a substance may cause enzyme induction in vivo by stress-induced adrenal secretion and the hormone-induced mechanism, adrenalectomized rats were used to determine the specificity of the substrate-induced enzyme response. The same analogues of tryptophan also were tested as substrates, inhibitors, and stabilizers of the cell-free enzyme preparation to determine whether there was any relation between the inducing activity of a substance and its affinity for the enzyme.

METHODS

Compounds—Dr. Karl Pfister, Merck and Company, Inc. gave us the sample of α-methyl-DL-tryptophan. Dr. R. Snyder, Urbana, Illinois, gave us the samples of DL-tryptazan, 5-methyl-DL-tryptophan, and 6-methyl-DL-tryptophan. N"-Acetyl-L-tryptophan was synthesized by the method of du Vigneaud and Sealock (4). The remaining compounds were purchased from commercial sources.

Induction—Adult albino rats were adrenalectomized 4 to 6 days before use and were maintained on daily subcutaneous doses of 0.5 mg of deoxycorticosterone acetate. This treatment prevented the early death of the rats after tryptophan injection (1). The test compounds were given intraperitoneally as suspensions in 0.9% NaCl at a dose of 50 μg in 1 ml for each 100 g body weight. Controls received 0.9% sodium chloride injections. The animals were killed four hours after injection and the livers removed for immediate assay of tryptophan pyrrolase.

Enzyme Assay—The assay of tryptophan pyrrolase was the same as described earlier. It consisted of the measurement of kynurenine formed during a 1 hour incubation with two concentrations of liver homogenate (5), and with the addition to the system of catalase, glucose, and glucose oxidase (2). Activity was referred to the dry weight of the homogenate.

Similar assays on fresh homogenates of liver from normal rats were used to determine the affinity of the tryptophan analogues for the enzyme. In the tests for substrate activity, the analogues replaced L-tryptophan in the incubation mixture. In the tests for inhibitor activity, they were added to the tryptophan-containing system at twice the concentration of L-tryptophan. In the tests for stabilization of the enzyme, the compounds in the usual substrate concentrations were incubated for 30 minutes with the otherwise complete system before L-tryptophan was added for the 1-hour assay. Comparisons were made in each instance with the activity on L-tryptophan determined at the same time with the same enzyme preparation.

RESULTS

The levels of tryptophan pyrrolase induced by the injection of the test compounds are shown in Table I (second column) and compared with the evidence for affinity of these compounds with the enzyme in tests in vitro (last 4 columns).

Inducers—Of the analogues tested in the adrenalectomized animals, β-tryptophan and α-methyl-DL-tryptophan were potent inducers, though less effective than L-tryptophan, and N"-acetyl L-tryptophan had moderate inducing activity. A separate experiment showed that one of the ineffective compounds, 5-methyltryptophan, administered in the same dosage 2 hours before injection of hydrocortisone, did not alter the induction of tryptophan pyrrolase by hydrocortisone. The enzyme level after hydrocortisone was equivalent to 25.0 and after pretreatment with 5-methyltryptophan to 26.5 μmoles per g dry weight per hour. 5-Methyltryptophan also did not induce tryptophan pyrrolase in Pseudomonas (6). In bacteria, 5- and 6-methyl-tryptophan and tryptazan inhibited growth and the last two inhibited formation of inducible enzymes (7, 8).

Substrates—5-Methyl- and 6-methyl-DL-tryptophan were converted by the liver enzyme to products with maximum absorption at 360 μg, which were believed to be analogues of kynurenine. Ichihara et al. (10) previously observed that liver homogenates oxidized 5-methyltryptophan to a kynurenine-like product. The tryptophan pyrrolase of Pseudomonas does not oxidize 5-methyltryptophan (11). N"-Acetyl-L-tryptophan was very slightly converted to kynurenine by crude liver enzyme preparations, but only after its hydrolysis to free tryptophan. This reaction was eliminated by partial purification of the
tryptophan pyrrolase (see also (9)). None of the other compounds tested were oxidized by the enzyme.

The lack of oxidation of α-methyl-DL-tryptophan supports the conclusion tentatively reached by Sourkes and Townsend (3), who reported that, in a few experiments, the amount of material with absorption at 360 nm increased during the reaction. However, extensive study of this point by them and by us failed to demonstrate any detectable formation of α-methylkynurenine.

Inhibitors—D-L-Tryptazan, 5-methyl-DL-tryptophan, and α-methyl-DL-tryptophan were decreasingly inhibitory in that order. Similar mild inhibitions of the *Pseudomonas* tryptophan pyrrolase by 5-methyltryptophan (12) and of the rat liver enzyme by α-methyl-DL-tryptophan (3) have been recorded previously. The latter inhibition appeared to be competitive. None of the compounds tested showed the strong inhibitory action of 5-hydroxytryptophan on the *Pseudomonas* enzyme (12) or on the liver enzyme (13).

Stabilizers—In the absence of its substrate there is rapid inactivation of the pyrrolase when incubated at 37° as a liver homogenate (9), as a purified preparation (9), or in slice (2), and when stored as a homogenate for longer periods at 2° (14). L-Tryptophan stabilizes the enzyme under these conditions by an unknown mechanism (15). As shown for two representative experiments in Table I, besides L-tryptophan, only α-methyl-DL-tryptophan stabilized the enzyme significantly, and it was as effective as L-tryptophan. Sourkes and Townsend (3) discovered this stabilizing effect of α-methyl-DL-tryptophan on tryptophan pyrrolase. Very slight stabilizing effects were observed with most of the other compounds listed as well as with a number of other indole compounds.

### DISCUSSION

Since the stress of injection of compounds can induce the liver tryptophan pyrrolase by activating adrenal hormone secretion, the specificity of substrate-type induction can be tested only in adrenalectomized animals. The present experiments demonstrate that several tryptophan analogues have inducing actions of the substrate type. The inducing activity of two of these, D-tryptophan and N*-acetyl-L-tryptophan, may possibly be referable to their conversion in the animal to L-tryptophan. Both of these compounds can replace tryptophan in the diet of the growing rat (10, 11), but it is not known if their rate of conversion to L-tryptophan is sufficient to account for the inducing actions observed. There are indications that in man D-tryptophan and N*-acetyl-L-tryptophan may be oxidized directly by reactions like that catalyzed by tryptophan pyrrolase. Administration of these compounds to man results in the excretion respectively of n-kynurenine and N*-acetylkynurenine (18, 19). However, there is no evidence that the intact rat or the cell-free rat enzyme oxidizes these compounds directly. The two compounds showed no evidence for affinities with the enzyme in the present experiments and their role as inducers, direct or indirect, remains to be clarified. It is unlikely that the other effective inducer, α-methyl-DL-tryptophan, is converted to tryptophan to an appreciable extent by the rat.

It is now recognized that an inducer of a microbial enzyme need not be a substrate, or even an inhibitor of that enzyme. The present results establish this fact for the liver tryptophan pyrrolase induction. The three tryptophan analogues that were effective inducers were not oxidized by the enzymes. Slight activity as a substrate (5- and 6-methyltryptophans) or as an inhibitor (5-methyltryptophan and tryptazan) was not sufficient to permit induction. The ability to stabilize the enzyme *in vitro* at 37°, shown only by L-tryptophan and α-methyl-DL-tryptophan, stands out as a possible correlative of the inducing activity. This correlation is valid only if it is assumed that n-tryptophan and N*-acetyl-L-tryptophan may be oxidized directly by reactions like that catalyzed by tryptophan pyrrolase. The present results establish this fact for the liver tryptophan pyrrolase induction. The three tryptophan analogues that were effective inducers were not oxidized by the enzymes. Slight activity as a substrate (5- and 6-methyltryptophans) or as an inhibitor (5-methyltryptophan and tryptazan) was not sufficient to permit induction. The ability to stabilize the enzyme *in vitro* at 37°, shown only by L-tryptophan and α-methyl-DL-tryptophan, stands out as a possible correlative of the inducing activity. This correlation is valid only if it is assumed that n-tryptophan and N*-acetyl-L-tryptophan may be oxidized directly by reactions like that catalyzed by tryptophan pyrrolase.

### Table I

<table>
<thead>
<tr>
<th>Compounds</th>
<th><em>In vivo</em> Activity induced</th>
<th><em>In vivo</em> Oxidation as substrate</th>
<th><em>In vivo</em> Inhibition of α-tryptophan oxidation</th>
<th><em>In vitro</em> Stabilization after 30 minutes at 37°</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmoles/g dry wt/hr</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>26.1 (5)</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>D-Tryptophan</td>
<td>19.2 (4)</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>α-Methyl-DL-tryptophan</td>
<td>19.0 (3)</td>
<td>0</td>
<td>14</td>
<td>99</td>
</tr>
<tr>
<td>N*-Acetyl-L-tryptophan</td>
<td>10.0 (6)</td>
<td>0*</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>D-L-Tryptazan</td>
<td>5.5 (2)</td>
<td>0</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>5-Methyl-DL-tryptophan</td>
<td>5.2 (2)</td>
<td>10</td>
<td>29</td>
<td>45</td>
</tr>
<tr>
<td>6-Methyl-DL-tryptophan</td>
<td>3.4 (2)</td>
<td>10</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>0.9% Sodium chloride solution</td>
<td>6.2 (6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Slight activity observed in homogenates was eliminated by purification of the enzyme.

† Activity remaining after 30 minutes' incubation without addition of any compound.
ship between the abilities of d-tryptophan and other analogues to induce the enzyme in vivo and to stabilize it at 4°C in vitro.

It is necessary to point out that the stabilization in vitro of tryptophan pyrrolase by L-tryptophan and by α-methyl-dL-tryptophan indicates only some specific type of interaction in vitro with or affinity for the enzyme. So far as is known, it is not a mechanism by which the induced levels in vivo of the enzyme could arise. Dubnoff and Dimick (14) suggested that stabilization by administered tryptophan of the tryptophan pyrrolase being continuously synthesized in the liver might account for accumulation of the high levels of enzyme found in treated rats. However, they observed only the stabilization in vitro against inactivation which occurs in the absence of tryptophan during incubation or prolonged storage of cell-free enzyme preparations. There is no evidence available for a similar stabilization in vivo. Extraction of the enzyme from the tissue in the absence of added tryptophan does not entail losses of activity, and extraction in the presence of added tryptophan does not result in higher activity. The lag period between tryptophan administration and enzyme accumulation indicates that a slower process than stabilization by tryptophan produces the high enzyme activities. Time curves relating the levels of free tryptophan and of tryptophan pyrrolase in the liver during induction (2) show that free tryptophan reaches its maximum level in the liver while the enzyme level is still normal, and that the tryptophan level is reduced to normal by the time the enzyme activity becomes maximal. Elevated enzyme levels then persist for some hours while the free tryptophan level is normal. The induction of tryptophan pyrrolase in liver slices also separated the two phenomena of induction and stabilization (2). Thus, stabilization of the enzyme in incubated slices required only the addition of tryptophan, whereas induction of a net increase of enzyme in slices required tryptophan and also a complex medium of the type adequate to support protein synthesis.

SUMMARY

1. d-Tryptophan, α-methyl-dL-tryptophan, and Nω-acetyl-L-tryptophan showed substrate-type induction of liver tryptophan pyrrolase in adrenalectomized rats. 6-Methyl- and 5-methyl-dL-tryptophans and DL-tryptazan were ineffective as inducers in vivo although the first two were slowly oxidized by preparations of the enzyme, and the last two slightly inhibited the enzymic oxidation of tryptophan.

2. Although α-methyl-dL-tryptophan was an inducer, it was not a substrate of the enzyme. It showed some affinity for the enzyme, manifested by mild inhibitory action and by high activity in stabilizing the enzyme in vitro. A stabilization effect does not appear a likely explanation for the ability of α-methyl-dL-tryptophan (or of L-tryptophan) to induce the accumulation of the enzyme in vivo.

REFERENCES

The Specificity of Tryptophan Analogues as Inducers, Substrates, Inhibitors, and Stabilizers of Liver Tryptophan Pyrrolase
Morton Civen and W. Eugene Knox


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