Reactivities and Specific Inhibitory Effects of a 1,3,4-Thiadiazolo[3,2-a]pyrimidine on SH Enzymes†

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We studied the reactivity of 2-ethylsulfonyl-7-methyl-5//-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (TPSO2-2) with some amino acids, SH, OH, and histidine enzymes. TPSO2-2 reacted with L-cysteine in high yield (80~90%), but with glycine and L-serine in low yield (below 10%). To clarify the relative reactivity of TPSO2-2, its reaction with L-cysteine in the presence of ethanol and diethylamine was examined. TPSO2-2 reacted only with the SH group of L-cysteine, not with ethanol nor diethylamine. To elucidate the reactivity of TPSO2-2 toward enzymes, the inhibitory effects of TPSO2-2 on some enzymes containing cysteine, serine, or histidine in the active center were investigated. TPSO2-2 showed high inhibitory effects on SH enzymes, such as yeast alcohol dehydrogenase, glutamate dehydrogenase, and hexokinase, but no effect on trypsin, which has serine, or catalase, with histidine in the active center. TPSO2-2 appeared to be a specific inhibitor of SH enzymes.

Some derivatives of 1,3,4-thiadiazolo[3,2-a]pyrimidine, a purine analog, were shown to be cytotoxic to Ehrlich ascites tumor cells (E-cells) and other experimental cells in growing cultures.1) TPSO2-2, the most active compound in this series, especially inhibited RNA synthesis by DNA-dependent RNA polymerase of E-cells, but scarcely inhibited that of E. coli.2) In order to elucidate the mechanism of the inhibition, the chemical reactions of TPSO2-2 with SH compounds in vitro were investigated, and it was found that TPSO2-2 readily reacted with those compounds at the 2-position carbon atom of thiadiazolopyrimidine. Therefore, TPSO2-2 appeared to directly inactivate the enzyme by alkylation of the SH group.3) Another biochemical activity of TPSO2-2, the inhibition of respiration, appeared to be a possible cause of its bactericidal activity, but it was thought to be only a minor factor for its cytotoxicity against E-cells.4) Both the electron-withdrawing sulfonyl group and pseudopurine skeleton appeared to be responsible for the biological activities and chemical properties of TPSO2-2.5)

In this paper, reactions of TPSO2-2 with some amino acids and its inhibitory effects on some enzymes were investigated. TPSO2-2 reacted with SH compounds preferentially, and appeared to be specific inhibitor of SH enzymes.

EXPERIMENTAL

Reaction of TPSO2-2 with t-serine or glycine. To a mixture of 0.5 g of TPSO2-2 and 0.3 g of t-serine in 30 ml of acetone-water (1 : 1, v/v), 0.02 ml of diethylamine was slowly added with stirring, and the mixture was kept at room temperature for 3 hr and extracted with chloroform to remove unreacted TPSO2-2. The aqueous layer was concentrated in vacuo and the residue was adsorbed on an Amberlite CG-120 column (2.2 x 17 cm), and eluted with pH 3.5 sodium citrate buffer (500 ml), distilled water (500 ml), and 2N NH4OH solution (300 ml) in this order. The distilled water fraction gave yellow needles on evaporation, and this compound was negative to ninhydrin. Yield, 0.04 g (7.7%), mp 285°C. Anal. Found: C, 40.27; H, 3.36; N, 20.98%. Calcd. for C9H10O4N4S: C, 40.00; H, 3.36; N, 20.98%.

† Studies on Biologically Active Thiadiazolopyrimidines. Part VI. For Part V, see ref. 5.

Abbreviations: TPSO2-2, 2-ethylsulfonyl-7-methyl-5//-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one; E-cells, Ehrlich ascites tumor cells; y-ADH, yeast alcohol dehydrogenase; ID50, 50% inhibitory dose.
Effect of TPSO2-2 on hexokinase. The activity of hexokinase (EC 2.7.1.1) (purchased from the Boehringer Mannheim Yamanouchi Co.) was assayed according to Schulze.\(^8\) One ml of 0.02% hexokinase in 0.1m triethanolamine buffer, pH 7.6, was incubated with 0.5 ml of TPSO2-2 at 25°C for 1 hr. This mixture (0.02 ml) was transferred to a quartz cell, to which were added 1.27 ml of the above buffer, 1.2 ml of 0.55m D-glucose in the same buffer, 0.20 ml of 0.1 m MgCl\(_2\), 0.10 ml of 81mM ATP, 0.20 ml of 11 mM NADP and 0.01 ml of 0.04% glucose-6-phosphate dehydrogenase. Absorbance readings at 340 nm (2-min intervals) and the calculation of inhibition (%) were made in the same manner as the case of trypsin.

Effect of TPSO2-2 on fumarase. The activity of fumarase (L-malate hydro-lyase, EC 4.2.1.2) (purchased from the Boehringer Mannheim Yamanouchi Co.) was assayed according to Massey.\(^9\) One ml of 0.0001% fumarase in 0.1% serum albumin solution was incubated with 0.5 ml of TPSO2-2 at 25°C for 1 hr. This mixture (0.02 ml) was transferred to a quartz cell, to which was added 3.0 ml of 50 mM malate in 0.1m phosphate buffer, pH 7.6. Absorbance readings at 240 nm (2-min intervals) and the calculation of inhibition (%) were made in the same manner as the case of trypsin.

RESULTS AND DISCUSSION

Reaction of TPSO2-2 with l-serine or glycine

It was reported previously that TPSO2-2 reacted with L-cysteine and other SH compounds at the 2-position carbon atom of thiadiazolopyrimidine in high yield.\(^3,5\) In order to clarify the reactivity of TPSO2-2, reactions of TPSO2-2 with other amino acids such as L-serine and glycine were then examined. TPSO2-2 was treated with L-serine or glycine in a diethylamine solution, and the reaction products were purified by Amberlite CG-120 column chromatography. Yellow needles were obtained in low yields and the compounds were negative to ninhydrin. The results of the elemental analyses and the in
Specific Inhibitions of SH Enzymes by Thiadiazolopyrimidine

Fig. 1. Reactions of TPSO2-2 with Various Amino Acids.

**TABLE I. EFFECT OF TPSO2-2 ON AN OH ENZYME (TRYPSIN)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID50 (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPSO2-2</td>
<td>&gt;3.9 x 10^{-3}</td>
</tr>
<tr>
<td>Trypsin inhibitor</td>
<td>&lt;2.2 x 10^{-5}</td>
</tr>
<tr>
<td>(from soybeans)</td>
<td>(100%)</td>
</tr>
</tbody>
</table>

* Inhibition (%) at the indicated concentration.

**TABLE II. EFFECT OF TPSO2-2 ON A HISTIDINE ENZYME (CATALASE)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID50 (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPSO2-2</td>
<td>&gt;3.5 x 10^{-4}</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>&gt;5.0 x 10^{-4}</td>
</tr>
</tbody>
</table>

* Inhibition (%) at the indicated concentration.

Infrared spectra showed that those compounds were identical with N-1,3,4-thiadiazolo[3,2-a]pyrimidinyl serine and N-1,3,4-thiadiazolo[3,2-a]pyrimidinyl glycine. Another reaction product of TPSO2-2 with serine was positive to ninhydrin, and the structure was identical with O-1,3,4-thiadiazolo[3,2-a]pyrimidinyl serine. Furthermore, a large amount of unreacted TPSO2-2 was recovered in both cases.

TPSO2-2 reacted readily with L-cysteine in high yield, 80 ~ 90%, as reported previously, but with L-serine and glycine in low yield, below 10%. This evidently indicates that the carbon atom at the 2-position of TPSO2-2 prefers to react with SH groups, compared with NH2 or OH groups. The reaction schemes are shown in Fig. 1.

**Reaction of TPSO2-2 with L-cysteine in ethanol**

TPSO2-2 was demonstrated to react with alcohol at the 2-position carbon atom in the presence of diethylamine.3) To clarify the relative reactivity of TPSO2-2, reactions of TPSO2-2 with SH compounds in the presence of OH compounds were examined. L-Cysteine and ethanol were used as the representatives of SH and OH compounds. TPSO2-2 reacted only with L-cysteine to give S-1,3,4-thiadiazolo[3,2-a]pyrimidinyl cysteine in the presence of ethanol and diethylamine. This fact also indicates that the carbon atom at the 2-position of TPSO2-2 prefers to react with SH groups, and the result is consistent with Pearson's concept11) that the 2-position carbon atom of TPSO2-2 is a soft acid, and reacts easily with an SH group which is a softer base than OH or NH2 group.

**Effect of TPSO2-2 on an OH enzyme**

Since TPSO2-2 was demonstrated to react with alcohol and OH group of L-serine although in low yield, it might inhibit OH enzymes containing serine in the active center. Its activity toward OH enzymes was then investigated, and trypsin was used as an example of OH enzymes. TPSO2-2 showed no effect even at a high concentration (3.9 x 10^{-3} m) as shown in Table I. Trypsin inhibitor from soybeans, used as a positive control, showed a strong inhibitory activity and the
Table III. Effects of TPSO₂⁻² on SH Enzymes

<table>
<thead>
<tr>
<th>Compound</th>
<th>ADH ID₅₀ (m)</th>
<th>GLDH ID₅₀ (m)</th>
<th>Hexokinase ID₅₀ (m)</th>
<th>Fumarase ID₂₀ (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPSO₂⁻²</td>
<td>1.9 x 10⁻⁵</td>
<td>1.7 x 10⁻⁴</td>
<td>1.2 x 10⁻⁴</td>
<td>1.0 x 10⁻⁴</td>
</tr>
<tr>
<td>PCMB</td>
<td>3.0 x 10⁻⁵</td>
<td>3.0 x 10⁻⁵</td>
<td>3.8 x 10⁻⁵</td>
<td>—</td>
</tr>
<tr>
<td>IAA</td>
<td>—</td>
<td>5.0 x 10⁻⁴</td>
<td>—</td>
<td>4.2 x 10⁻²</td>
</tr>
</tbody>
</table>

¹ Yeast alcohol dehydrogenase.
² Glutamate dehydrogenase.
³ p-Chloromercuribenzoic acid.
⁴ Monoiodoacetic acid.

concentration for the 50% inhibitory dose (ID₅₀) was below 2.2 x 10⁻⁵ m.

Effect of TPSO₂⁻² on a histidine enzyme

It was thought that TPSO₂⁻² might inhibit enzymes containing histidine in their active centers, because TPSO₂⁻² reacted with the amino groups of glycine and serine as described above. Its activity toward histidine enzymes was then investigated, and catalase was used as an example of histidine enzymes. TPSO₂⁻² was found to have no effect even at a high concentration (3.5 x 10⁻⁴ m), while ascorbic acid, used as a positive control, exerted some inhibitory activity as shown in Table II.

Effects of TPSO₂⁻² on SH enzymes

TPSO₂⁻² showed a high inhibitory effect on an SH enzyme, yeast alcohol dehydrogenase (y-ADH), and the ID₅₀ was 1.9 x 10⁻⁵ m.⁵ Then its activities toward some other SH enzymes, glutamate dehydrogenase, hexokinase, and fumarase, were examined. TPSO₂⁻² showed a strong inhibitory effect on them as shown in Table III, and its ID₅₀ on glutamate dehydrogenase was 1.7 x 10⁻⁴ m, on hexokinase 1.2 x 10⁻⁴ m, and its ID₂₀ (20% inhibitory dose) on fumarase 1.0 x 10⁻⁴ m. The inhibitory effect of TPSO₂⁻² on y-ADH was stronger than that of p-chloromercuribenzoic acid (PCMB), but the effects on glutamate dehydrogenase and hexokinase were a little weaker than those of PCMB. Inhibitory activities of TPSO₂⁻² on these enzymes were stronger than that of an alkylating SH enzyme inhibitor, monoiodoacetic acid. TPSO₂⁻² therefore appears to be a specific inhibitor of SH enzymes.

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REFERENCES