Peroxidizing環状イソイミド化合物の酵素による活性化

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Original Article

The Enzymatic Activation of Peroxidizing Cyclicisoimide: A New Function of Glutathione *S*-Transferase and Glutathione

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A herbicidal peroxidizer, *N*-(4-bromophenyl)-3, 4, 5, 6-tetrahydroisophthalimide was isomerized to *N*-(4-bromophenyl)-3, 4, 5, 6-tetrahydrophthalimide directly and rapidly in the presence of equine glutathione *S*-transferase (GST) with reduced glutathione (GSH), and was also converted into the imide *via* 4-bromophenyl-3, 4, 5, 6-tetrahydrophthalamic acid by hydrolysis. GST converts 5-(4-bromophenylimino)-3, 4-tetramethylene-1, 3, 4-thiadiazolidin-2-one into its isomer, 4-bromophenyl-1, 2-tetramethylene-1, 2, 4-triazolidin-3-one-5-thione, but hardly converts 5-(4-bromophenylimino)-3, 4-tetramethylene-1, 3, 4-thiadiazolidine-2-thione into 4-bromophenyl-1, 2-tetramethylene-1, 2, 4-triazolidine-3, 5-dithione. *N*-(4-Bromophenyl)-3, 4, 5, 6-tetrahydroisophthalimide and 5-(4-bromophenylimino)-3, 4-tetramethylene-1, 3, 4-thiadiazolidin-2-one strongly inhibit protoporphyrinogen oxidase activity after isomerization to their isomers. This finding, together with our previous results, reveals that GST catalyzes a quick activation of *N*-aryl-3, 4, 5, 6-tetrahydroisophthalimides and 5-arylimino-3, 4-tetramethylene-1, 3, 4-thiadiazolidin-2-ones in the presence of GSH by isomerization. Although GST has generally been described as a detoxifying enzyme in pesticide toxicology, the herbicide activation by the metabolic isomerization shown in this study is a new function of GST and GSH.

INTRODUCTION

Peroxidizing herbicides, such as cyclic imides and diphenyl ethers, cause membrane destruction in plants by inhibiting the membrane-bound protoporphyrinogen oxidase (protox, EC 1.3.3.4) competitively. This inhibition induces the accumulation of protoporphyrin IX which is sensitized by light with subsequent radical formation leading to degradation of cellular constituents with evolution of ethane.[1]

To establish a suitable weed control by peroxidizing herbicides, the selectivity of each peroxidizer should be found. Because protox is present in all photosynthetic plants, it should be necessary to study the differential metabolism of the peroxidizing herbicides which may influence their interaction with the target site in plants.

In a previous paper, we reported that 5-arylimino-3, 4-tetramethylene-1, 3, 4-thiadiazolidin-2-ones were isomerized to 4-aryl-1, 2-tetramethylene-1, 2, 4-triazolidin-3-one-5-thiones in the culture of *Echinochloa utilis* and *Scenedesmus acutus*, and by glutathione *S*-transferase (GST) before acting as strong peroxidizers.[2,3] 5-Arylimino-3, 4-tetramethylene-1, 3, 4-thiadiazolidine-2-thiones, however, were hardly converted into 4-aryl-1, 2-tetramethylene-1, 2, 4-triazolidine-3, 5-dithiones under the same conditions.[4] GST was reported as being involved in this conversion.[5] The major isomerizing factor was identified as the GST II form in corn seedlings.[6,7]

We already reported that *N*-aryl-3, 4, 5, 6-tetrahydroisophthalimides (isoimides) were rapidly hydrolyzed to *N*-aryl-3, 4, 5, 6-tetrahydrophthalamic acids and subsequently recyclized to *N*-aryl-3, 4, 5, 6-tetrahydrophthalimides (imides) in the culture of *E. utilis* and in buffer solution.[8] The conversion rate was faster in the culture of *E. utilis* than in buffer solution. So, it is reasonable to consider that GST also catalyzes the conversion of isoimides into imides.

In this paper, the enzymatic conversion of isoimides into imides and a new role of GST are reported.

MATERIALS AND METHODS

1. Chemicals

N-(4-Bromophenyl)-3, 4, 5, 6-tetrahydroisophthalimide (**1**), *N*-(4-bromophenyl)-3, 4, 5, 6-tetrahydrophthalimide (**2**), 5-(4-bromophenylimino)-3, 4-tetramethylene-

1, 3, 4-thiadiazolidin-2-one (**3**), 4-bromophenyl-1, 2-tetramethylene-1, 2, 4-triazolidin-3-one-5-thione (**4**), 5-(4-bromophenylimino)-3, 4-tetramethylene-1, 3, 4-thiadiazolidine-2-thione (**5**), 4-bromophenyl-1, 2-tetramethylene-1, 2, 4-triazolidine-3, 5-dithione (**6**) and 4-bromophenyl-3, 4, 5, 6-tetrahydrophthalamic acid (**7**) were synthesized according to previous procedures cited in Refs. 2, 4 and 8.

2. *Biological Tests*

Root growth inhibition of *E. utilis*, determination of chlorophyll decrease and ethane formation using autotrophic *S. acutus*, and inhibition of protoporphyrinogen oxidase prepared from corn (*Zea mays* cv. Anjou) etioplasts were performed according to the methods cited in Refs. 1, 2 and 9. Root growth inhibition, chlorophyll decrease, ethane formation and protox inhibition were expressed as pI_{50} (Ech), pI_{50} (Chl), pI_{50} (Eth), and pI_{50} (Protox), respectively.[1,2,9]

3. *Conversion Assay by Equine GST*

Isoimide (**1**) (0.1 mM), reduced glutathione (GSH) (0.1 mM) and 0.1 mg protein (=5–10 units)/ml of equine GST in 0.05 M potassium phosphate buffer (pH 6.8) were incubated for 2 hr at 30°C. Conversion was detected by a Shimadzu LC-6A HPLC system equipped with Senshu Pak ODS-1251-SK column (4.5ϕ×50 mm: Senshu Scientific Co., Tokyo, Japan). A solvent mixture of acetonitrile–distilled water (3 : 2, v/v) was used as the mobile phase (flow rate 1 ml/min) and the eluates were monitored by SSC-3000B UV detector (Senshu Scientific Co.) at 210 nm. The amounts equivalent to the peaks were expressed in percent of the peak areas of the compounds.

RESULTS AND DISCUSSION

As shown in Fig. 1a, with the reaction proceeding in buffer without GST, the decrease of isoimide (**1**) and the increase of both imide (**2**) and 4-bromophenyl-3, 4, 5, 6-tetrahydrophthalamic acid (amide acid) (**7**) began 3 min after start of the reaction. A rapid accumulation of the amide acid (**7**) started 10 min after start. At the end of incubation (2 hr), the percent of the amounts of the isoimide, the imide, the amide acid and 4-bromoaniline were 5.1, 52.3, 39.0 and 3.6%, respectively. The changes in the amount of the four compounds during the conversion experiment with equine GST present were almost the same as found in buffer solution without GST (Fig. 1b). Figure 1c shows the conversion of isoimide (**1**) with GST and GSH present. Isoimide (**1**) began to convert into imide (**2**) at the beginning of the incubation, and 49.4% of isoimide (**1**) were isomerized to the imide (**2**) already 1 min after start. The maximum isomerization (92.7%) of the isoimide was observed 10 min after start, and at this point, the amide acid (**7**) appeared in the test solution and decrease of the imide began. At the end of incubation, the percent of the amounts of the isoimide, the imide, the amide acid and 4-bromoaniline were 0, 56.8, 42.0 and 1.2%, respectively. These results indicate that GST with GSH isomerized the isoimide to the imide rapidly and directly. Without enzyme and cofactor, however, the isoimide was converted into the imide by hydrolysis and recyclization in buffer at pH 6.8, because the hydrolysis of the isoimide (**1**) and the accumulation of both imide (**2**) and amide acid (**7**) started at almost same time (see Fig. 2). This mechanism is supported by the finding that *N*-phenyl-3, 4, 5, 6-tetrahydrophthalamic acid is cyclized to *N*-phenyl-3, 4, 5, 6-tetrahydrophthalimide in neutral condition.[10] GST and GSH

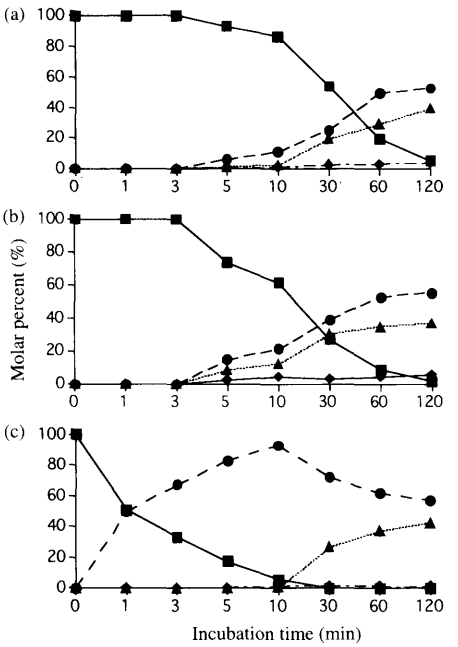


Fig. 1 Conversion of *N*-(4-bromophenyl)-3, 4, 5, 6-tetrahydroisophthalimide.
(a) In potassium phosphate buffer (pH 6.8). (b) In the presence of GST in phosphate buffer. (c) In the presence of GST and GSH in phosphate buffer. ■: *N*-(4-bromophenyl)-3, 4, 5, 6-tetrahydroisophthalimide (**1**), ●: *N*-(4-bromophenyl)-3, 4, 5, 6-tetrahydrophthalimide (**2**), ▲: 4-bromophenyl-3, 4, 5, 6-tetrahydrophthalamic acid (**7**), ◆: 4-bromoaniline.

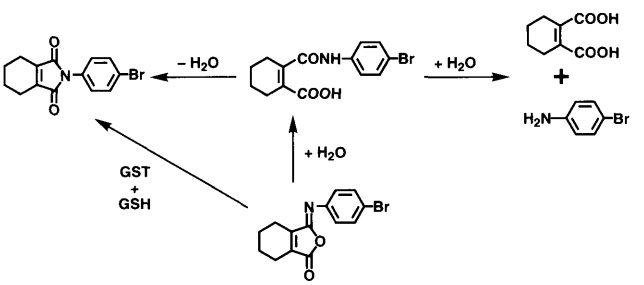
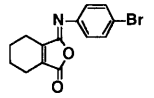
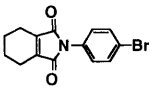
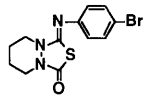
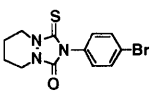
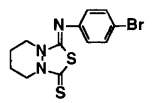
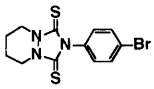


Fig. 2 Interconversion of isoimide, imide and amide acid.

Table 1 Phytotoxic activity of compounds tested.

	Compounds	pI_{50} (Ech)	pI_{50} (Chl)	pI_{50} (Eth)	pI_{50} (Protox)
1		6.09	5.65	6.40	7.37
2		6.04	5.91	6.47	7.55
3		6.60	6.57	6.43	5.30
4		6.79	6.46	6.57	8.00
5		6.61	6.83	6.26	6.13
6		7.18	7.43	7.53	8.14

See section *2* of "MATERIALS AND METHODS" for abbreviations.

acted only on the isoimide, but not on the amide acid in our experiment (data not cited).

Table 1 shows phytotoxic activities of the compounds (**1**)-(**6**). Inhibitory activity against protox, pI_{50} (Protox), of (**1**) and (**2**) were 7.37 and 7.55, respectively. But we believe that pI_{50} (Protox)=7.37 of the isoimide (**1**) means the pI_{50} (Protox) of imide (**2**) actually, because isoimide (**1**) isomerized to imide (**2**) very rapidly as shown in Fig. 1. The inhibitory activities of the isoimide (**1**) and the imide (**2**) against the root growth of *E. utilis* (pI_{50} (Ech)), chlorophyll decrease (pI_{50} (Chl)) and ethane formation (pI_{50} (Eth)) in *S. acutus* were very close, because both enzymatic isomerization and hydrolytic conversion of isoimide (**1**) into imide (**2**) were operative in the assay conditions (see Fig. 2). It has been reported that GST in corn and equine GST isomerized thiadiazolidine (**3**) to 4-bromophenyl-1, 2-tetramethylene-1, 2, 4-triazolidin-3-one-5-thione (triazolidine) (**4**) in the presence of SH compounds such as GSH and DTT.[3, 4, 6, 7] The pI_{50} (Protox) values of thiadiazolidine (**3**) and triazolidine (**4**) were 5.30 and 8.00, respectively. In this case, the enzymatic isomerization would work, but the reaction time was too short for isomerization. Other phytotoxic activities (pI_{50} (Ech), pI_{50} (Chl) and pI_{50} (Eth)) of these compounds were very close. These data indicate that the isomerization of (**1**) to (**2**) proceeds more easily than that of thiadiazolidine (**3**) to (**4**) by GST with GSH before inhibiting the protox activity. The inhibitory activity against protox of 4-bromophenyl-1, 2-tetramethylene-1, 2, 4-triazolidine-3, 5-dithione (triazolidine) (**6**) (pI_{50} (Protox)=8.14) was 100 times stronger than that of 5-(4-bromophenylimino)-3, 4-tetramethylene-1, 3, 4-thiadiazolidine-2-thione (thiadiazolidine) (**5**). Phytotoxic activities (pI_{50} (Ech), pI_{50} (Chl) and pI_{50} (Eth)) of triazolidine (**6**) were 4 to 19 times stronger than those of the thiadiazolidine (**5**). We previously mentioned these results and discussed that thiadiazolidine (**5**) was scarcely converted into triazolidine (**6**) by GST and GSH.[3, 4, 6]

Above mentioned findings together with the results in our previous paper give evidence that isoimides and thiadiazolidines isomerized to their corresponding imides and triazolidines by GST and GSH. The conversion rate was dependent on the core structure and the substituents in the *N*-aryl moiety. This means that GST activates isoimides and thiadiazolidines by isomerization.

Plant metabolism of herbicides usually results in deactivation, such as hydroxylation of aromatic rings in chlorsulfuron[11] and of methyl groups in chlorotoluron,[12] and sulfur oxidation in EPTC.[13] Conjugation with GSH catalyzed by GST is a very important detoxification mechanism in many plant tissues.[14] On the other hand, there are some notable examples where activation results. *N*-Dealkylation of sulfonylurea DPX-L-8747 gave a potent ALS inhibitors,[15] hydrolysis of bromoxynil octanoate released bromoxynil, a photosynthetic inhibitor,[16] and hydrolysis of imazamethabenz-methyl gave rise to imidazolinylbenzoic acid, an active ALS inhibitor,[17] and *N*-demethylation of metflurazone afforded the active inhibitor of phytoene desaturase, norflurazon.[18]

No reports have been found yet on metabolic activation of *N*-aryl-3, 4, 5, 6-tetramethyleneisophthalimides by GST with GSH. The metabolic isomerization and activation of *N*-aryl-3, 4, 5, 6-tetramethyleneisophthalimides and 5-arylimino-3, 4-tetramethylene-1, 3, 4-thiadiazolidin-2-ones are positive evidences of a new function of GST and GSH in plants.

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要　　約

Peroxidizing 環状イソイミド化合物の酵素による活性化：グルタチオン *S*-トランスフェラーゼとグルタチオンの新機能

佐藤幸治，Peter Böger，若林　攻

GST は還元型 GSH の存在下で *N*-(4-ブロモフェニル)-3, 4, 5, 6-テトラヒドロイソフタルイミド (**1**) を *N*-(4-ブロモフェニル)-3, 4, 5, 6-テトラヒドロフタルイミド (**2**) に，また，5-(4-ブロモフェニルイミノ)-3, 4-テトラメチレン-1, 3, 4-チアジアゾリジン-2-オン (**3**) を異性体であるトリアゾリジン型(**4**)に異性化した．しかしながら，5-(4-ブロモフェニルイミノ)-3, 4-テトラメチレン-1, 3, 4-チアジアゾリジン-2-チオン (**5**) は異性化されにくかった．(**1**) と (**3**) は異性化後にプロトポルフィリノーゲンオキシデースをより強く阻害した．GST は一般的に農薬の毒物学においては解毒化酵素として知られているが，上記のような異性化による活性化は GST と GSH の新しい機能である．