

# Occurrence of Mercaptopyruvate Sulfotransferase Activity in Photosynthetic Organisms

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Mercaptopyruvate sulfotransferase activity catalyzes the formation of pyruvate from mercaptopyruvate in the presence of suitable reagents as acceptor. It was detected in *Lemna minor*, *Pisum sativum*, *Spinacia oleracea*, *Chlorella fusca*, *Synechococcus 6301*, and *Rhodopseudomonas palustris*. Best activity was detected using dithioerythritol as a thiol reagent; good activity was obtained using mercaptoethanol, glutathione, mercaptopyruvate or sulfite as acceptor. The pH-optimum for the *Chlorella* mercaptopyruvate sulfotransferase was found around 9; the apparent  $K_m$  for mercaptopyruvate was determined to 2 mM and for dithioerythritol for 5 mM using crude *Chlorella* extracts. The role of this enzyme is discussed in relation to cysteine catabolism by photosynthetic organisms.

## Introduction

Plants and algae synthesize cysteine as the first sulfur-containing amino acid during assimilatory sulfate reduction [1]. Cysteine, however, is not only the amino acid needed for methionine and protein synthesis, but it might have other functions for the control of cellular metabolism as well. This was suggested recently for a control of the cellular thiol-disulfide level by the cysteine oxidation system described for *Synechococcus* [2] and *Chlorella* [3].

Evidence from algae and plants has demonstrated that elevated cysteine concentrations are toxic [4, 5] suggesting that the internal cysteine pool seems to be critical for a cell. Plants and algae can regulate the internal cysteine concentration by degrading cysteine to sulfide and emit this sulfide or oxidize it to sulfate [6–16]. The precise mechanism(s) of cysteine degradation are not fully understood so far. Therefore reactions leading to sulfide from cysteine or compounds related to cysteine have been analyzed. We will demonstrate in this publication for the first time that a mercaptopyruvate sulfotransferase activity is present in photosynthetic organisms. The relation of this enzyme to cysteine metabolism is discussed.

## Materials and Methods

### a) Organisms

*Spinacia oleracea* L. and *Pisum sativum* L. were grown in the greenhouse. *Lemna minor* L. was

maintained in axenic culture according to Brunold and Schmidt [17]. *Chlorella fusca* strain 211-8b from the algal collection of Göttingen was cultured as described earlier [18], and *Synechococcus 6301* (Pasteur Institute Paris) was grown as axenic culture as described earlier [19]. *Rhodopseudomonas palustris* cells grown on thiosulfate were a generous gift of Prof. Dr. Knobloch (Erlangen).

### b) Preparation of enzyme extracts

1) Plant systems: 2 g of leaf tissue was ground in a mortar using 4 ml of a buffer containing 0.1 M Tris-HCl buffer pH 8.0, 10 mM  $MgCl_2$ , and 10 mM mercaptoethanol. The crude extract was cleared by centrifugation and the supernatant was used as enzyme source. 2) Algal systems: 2 g of algal cells (wet weight) were suspended in 4 ml of the buffer mentioned above and broken in a french press at 12,000 psi (1 psi ~ 7 KPa) and cleared afterwards by centrifugation; the supernatant was used as the enzyme source. Extracts could be stored frozen without loss of activity for several days.

### c) Protein determination

The Coomassie blue method of Bradford [20] was used with the dye reagent preparation from Biorad. Bovine serum albumin was used as a reference.

### d) Measurements of mercaptopyruvate sulfotransferase activity

This enzyme activity was determined following the procedure according to Jarabak and Westley [21].

### e) Chemicals

Mercaptopyruvate was obtained from Fluka AG (Neu-Ulm, West-Germany); 2,4-dinitrophenylhydrazin and all other chemicals not mentioned were obtained from Merck (Darmstadt, West-Germany).

## Results

*Chlorella* extracts catalyzed the formation of pyruvate from mercaptopyruvate in the presence of various thiols. In order to define the conditions for the measurement of this mercaptopyruvate sulfotransferase activity, the following experiments were performed, using crude *Chlorella* extracts prepared as described in Materials and Methods.

### a) pH-optimum

Maximal pyruvate formation was achieved at pH-values above 9, as can be seen from the data of Fig. 1. We have used a pH of 9.0 for all experiments to be described.

### b) Protein dependence of the mercaptopyruvate sulfotransferase activity

The data of Fig. 2 demonstrate that good activity of this enzyme could be measured in the range of 1 mg of crude protein added. The reaction is linear at lower protein concentrations, however at higher protein concentrations the activity decreases, probably because the substrate becomes the limiting factor.

### c) $K_m$ -determination for mercaptopyruvate

The activity of the mercaptopyruvate sulfotransferase is dependent on the concentration of the substrate used, as shown in Fig. 3. From these data an apparent  $K_m$  for mercaptopyruvate was determined to 2 mM, using crude *Chlorella* extracts. These data were used to define the mercaptopyruvate concentration used in all further experiments. This concentration was set to 5 mM; higher concentrations increased the blanks, thus making this concentration a compromise between optimal substrate concentration and low blanks.

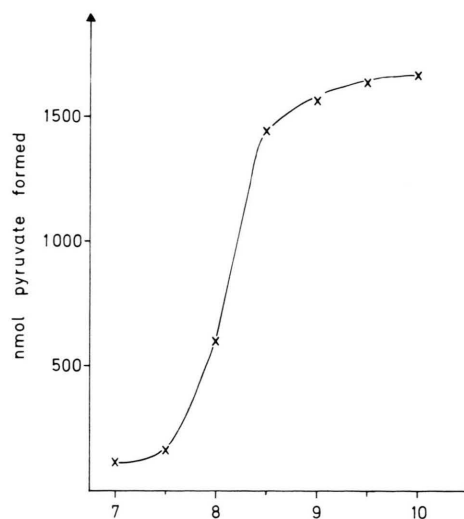


Fig. 1. pH-dependence of the mercaptopyruvate sulfotransferase activity from *Chlorella*. Conditions: Each vessel contained (in  $\mu\text{mol}$ ) in a total volume of 1 ml: Tris-HCl: 100 (pH varied as indicated); dithioerythritol: 10; mercaptopyruvate: 5; and 1.1 mg of crude *Chlorella* extract protein. Incubation for 30 min at 37 °C.

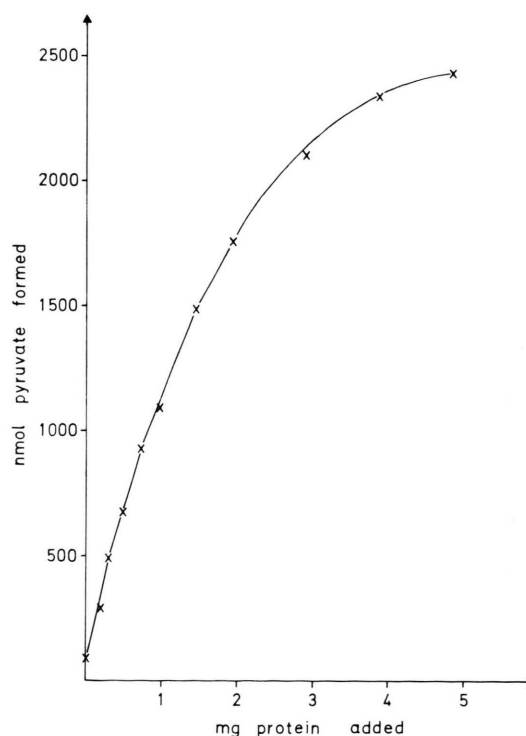


Fig. 2. Protein dependence of the mercaptopyruvate sulfotransferase activity from *Chlorella*. Conditions: As in Fig. 1, however the pH was constant at 9.0 and the protein concentration was varied as indicated.

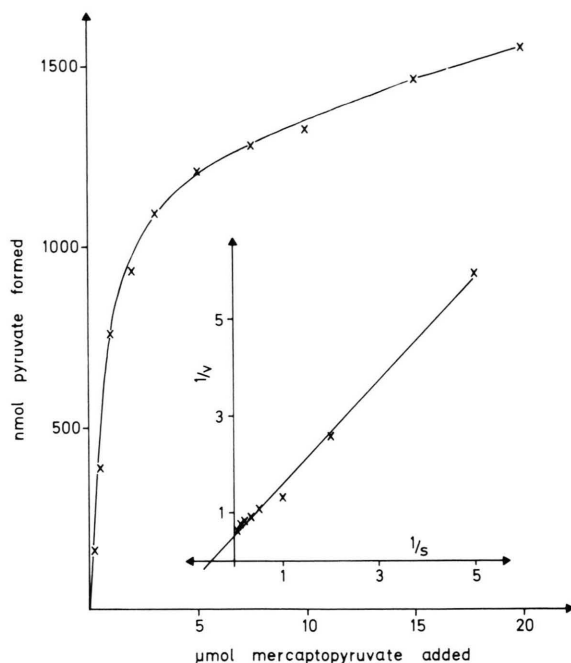


Fig. 3.  $K_m$ -determination for mercaptopyruvate. Conditions: As in Fig. 1, however the pH was kept constant at 9.0 and mercaptopyruvate was varied as indicated.

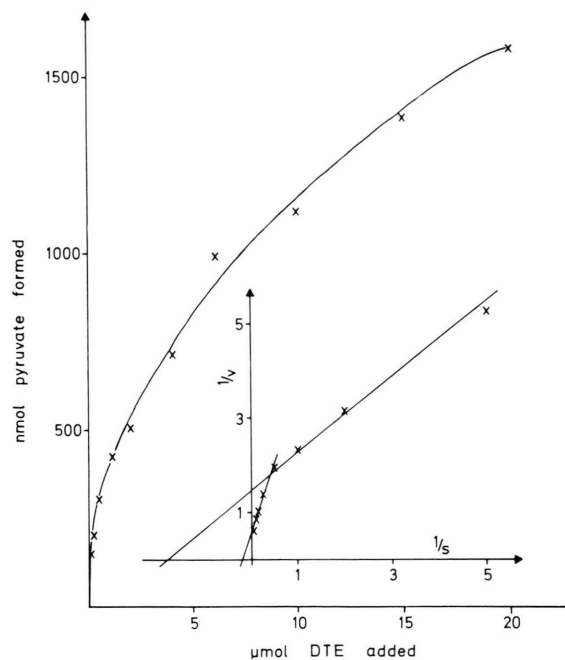


Fig. 4.  $K_m$ -determination for dithioerythritol. Conditions: As in Fig. 1, however the pH was constant at 9.0 and the dithioerythritol concentration was varied as indicated.

#### d) $K_m$ -determination for dithioerythritol

Addition of dithioerythritol to the assay system enhanced the mercaptopyruvate sulfotransferase activity as shown in Fig. 4. These data do not follow, however, simple Michaelis-Menten kinetics, since plotting of these data according to Lineweaver and Burk resulted in a broken line (insert of Fig. 4) indicating two different  $K_m$  areas, one about 0.56 mM and one at about 5 mM. It suggests that dithioerythritol could have a dual function leading to an activation of the enzyme besides its function as an acceptor. For our normalized conditions a concentration of 10 mM dithioerythritol was used, since higher thiol concentrations have been found to be inhibitory, if other thiol compounds were used.

#### e) Distribution of mercaptopyruvate sulfotransferase activity and comparison of certain sulfur acceptors

Crude extracts from higher plants (*Spinacia*, *Lemna*, *Pisum*), one green alga (*Chlorella fusca*), one cyanobacterium (*Synechococcus* 6301), and one

phototrophic bacterium (*Rhodospseudomonas palustris*) were analyzed for mercaptopyruvate sulfotransferase activity using different thiol acceptors. These data are summarized in Table I. For comparison we have normalized the data obtained with dithioerythritol as sulfur acceptor to 100% and calculated the other data as percentage of the dithioerythritol values. It is evident from these data that mercaptopyruvate sulfotransferase activity is detected in each species analyzed so far and the dithioerythritol-coupled rates are in the range of  $\mu\text{mol pyruvate formed per mg protein and hour}$  (*Lemna*: 6.51; *Spinacia*: 2.11; *Pisum*: 1.77; *Chlorella*: 1.55; *Synechococcus*: 3.97; and *Rhodospseudomonas*: 5.56). In all cases dithioerythritol is the best sulfur acceptor; the biological monothiol glutathione is active too, whereas cysteine supports low activity. Sulfite is an acceptor for this reaction and we could demonstrate the formation of thiosulfate (data not shown). Note, that further addition of mercaptopyruvate enhanced this activity, demonstrating that this compound is a donor and an acceptor for this reaction. Cyanide, thiocyanate, and thiosulfate are more or less inactive.

Table I. Acceptor specificity for mercaptopyruvate sulfotransferase.

Acceptor added	<i>Lemna minor</i>	<i>Pisum sativum</i>	<i>Spinacia oleracea</i>	<i>Chlorella fusca</i>	<i>Synechococcus 6301</i>	<i>Rhodospseudomonas palustris</i>
None	10	16	19	8	7	13
Dithioerythritol	100	100	100	100	100	100
Cysteine	7	28	19	13	10	8
Gluatathione	22	41	41	28	37	43
Mercaptoethanol	34	91	51	39	52	80
Mercaptopyruvate	19	54	36	31	28	51
Sulfite	14	17	15	13	27	75
Thiosulfate	2	12	3	9	3	13
Potassiumcyanide	1	24	9	14	14	27
Sodiumthiocyanate	0.5	11	14	11	16	16

Conditions: Each vessel contained (in  $\mu\text{mol}$ ) in a total volume of 1 ml: Tris-HCl pH 9.0: 100; mercaptopyruvate: 5; acceptor: 10; and protein as indicated below. The reaction was stopped after 30 min at 37 °C and analyzed for pyruvate formed. The following amounts of protein were added ( $\mu\text{mol}$ s pyruvate formed using DTE = 100): *Lemna*: 0.79 mg (2.57); *Pisum* 2.53 mg (2.24); *Spinacia* 1.32 mg (1.39); *Chlorella* 1.12 mg (0.879); *Synechococcus* 0.84 mg (1.70); *Rhodospseudomonas* 0.8 mg (2.22).

## Discussion

Plants and algae are capable to degrade cysteine to inorganic sulfide and to oxidize it to sulfate as discussed in the introduction chapter leading to a futile sulfur cycle [22]. Animals degrade cysteine either by oxygenation to cysteine sulfinic acid or transaminate it to mercaptopyruvate which is further metabolized to thiosulfate and pyruvate by a mercaptopyruvate sulfotransferase using sulfite as acceptor [23]. So far, evidence for the presence of mercaptopyruvate sulfotransferase activity is missing from plants and algae; Westley [23] stated in his recent review that this enzyme could be detected only in bacteria, fungi, and animals.

A search for this enzyme activity was initiated by our observation that *Chlorella* and spinach cells did not contain a L-cysteine specific desulfhydrase activity, suggesting that other mechanisms might be involved in cysteine degradation. One possible mechanism was demonstrated by the isolation of D-cysteine specific desulfhydrases from *Spinacia* and *Chlorella* [14, 15]; however, a racemase converting L-cysteine to D-cysteine was detected only by indirect methods so far. Another possible pathway for L-cysteine degradation would be comparable to animal systems implying that mercaptopyruvate is an intermediate for cysteine catabolism. This would be possible only, if a mercaptopyruvate sulfotransferase activity is present in plants and algae, and this had not been demonstrated so far [23]. The data of this publication demonstrate for the first time the

presence of mercaptopyruvate sulfotransferase activity in photosynthetic organisms.

The catalytic properties of the plant type mercaptopyruvate sulfotransferase have been determined using *Chlorella* extracts. The final conditions to measure this activity are a pH of 9.0; a mercaptopyruvate concentration of 5 mM and a dithioerythritol concentration of 10 mM. The analysis of different thiol reagents clearly demonstrated that dithioerythritol is the best sulfur acceptor leading to pyruvate, oxidized dithioerythritol and sulfide.  $K_m$ -determinations of dithioerythritol lead to two different  $K_m$ -values suggesting an apparent  $K_m$  of 0.56 and 5 mM. It is suggested that the enzyme can be activated by thiols as observed for the liver enzyme [24]. Pyruvate was determined using di-nitrophenylhydrazin. Determination of sulfide using the methylene-blue method leads to erroneous results since mercaptopyruvate is degraded non-enzymatic at acidic conditions yielding sulfide. It is evident from the data of Table I that cyanide is less effective compared to dithioerythritol; cyanide probably reacts directly with mercaptopyruvate forming a cyanohydrin adduct thus quenching the substrate [24]. Mercaptopyruvate sulfotransferase from plants and algae used mercaptoethanol as an acceptor; this clearly distinguished this reaction from thiosulfate reductase activities, where mercaptoethanol is not a sulfur acceptor [25]. Addition of sulfite leads to the formation of thiosulfate using the method of Sörbo [26]; therefore this enzyme activity could be active in mercaptopyruvate degra-

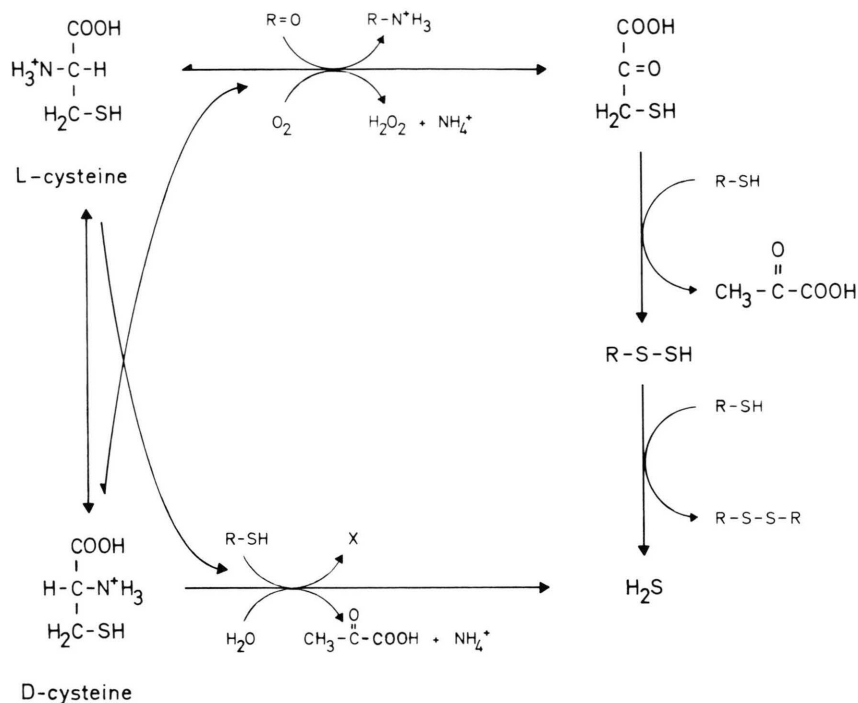


Fig. 5. Pathways for L- and D-cysteine degradation in photosynthetic organisms.

dation to pyruvate and thiosulfate as suggested for animals. Thiosulfate formed would then be cleaved by the thiosulfate reductase system [25] leading to sulfide and sulfite. It is clear that mercaptopyruvate itself is a donor and acceptor for this reaction using plant and algal systems; this was shown previously for the bovine kidney system [21, 27]. DTE was preferred as sulfur acceptor because the activity is enhanced and the blanks are considerably lower due to the thiol reducing capacity of dithioerythritol.

The evidence given in this publication suggests that mercaptopyruvate could be an intermediate in cysteine degradation to sulfide and elemental sulfur as suggested previously for *Chlorella* [28]. The following reactions summarized in Fig. 5 could be involved in cysteine catabolism in plants.

Mercaptopyruvate could either be generated by a transaminase reaction from cysteine and a suitable  $\alpha$ -keto acid or by coupling to an amino acid oxidase; these reactions can be coupled principally either to D- or L-cysteine. Mercaptopyruvate formed is used as a sulfur donor leading to thiosulfate if sulfite is used as an acceptor or to a persulfide if a thiol is used, with further reduction in both cases to free sulfide. Formation of zero valency sulfur can be

explained by this system as well as suggested by Krauss *et al.* [28] for *Chlorella*. Thus production of sulfide, ammonia, and pyruvate from cysteine could be a coupled reaction not catalyzed by a cysteine lyase. L-cysteine lyase activity was detected in *Acacia* species [29, 30]; in tobacco [11], and in the *Cruciferae* family [16], and we could detect L-cysteine lyase activity in this family as well. We did not find this activity however in spinach, *Lemna*, *Chlorella*, and *Synechococcus*; therefore we were searching for alternative possibilities. We detected D-cysteine specific lyase activities in plants and algae [14, 15], however evidence for a racemase converting L-cysteine to D-cysteine is obtained so far only by indirect methods. Since in the D-cysteine lyase reaction non-identified components could be found besides pyruvate and  $\text{NH}_4^+$  (unpublished), we have indicated this by the formation as X as unknown product.

Clearly, different mechanisms for L-cysteine catabolism have been suggested and could be realized in plants and algae. The precise determination of the mechanism(s) operative *in vivo* should be analyzed studying the regulation of the possible pathways involved in cysteine degradation.

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