The decarboxylation of 3-mercaptopyruvate to 2-mercaptoacetate

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Rat brain mitochondria were found to convert 3-mercaptopyruvate to 2-mercaptoacetate in the presence of NAD^+, coenzyme A and thiamin pyrophosphate. The overall reaction probably consists of an oxidative decarboxylation of 3-mercaptopyruvate with 2-mercaptoacetyl CoA as a product which is then hydrolyzed to 2-mercaptoacetate by acyl CoA hydrolase

3-Mercaptopyruvate *2-Mercaptoacetate* *α-Ketoacid decarboxylase* *Acyl CoA hydrolase*

1. INTRODUCTION

Small quantities of 2-mercaptoacetate as its mixed disulfide with cysteine are excreted in normal human urine [1] but the metabolic origin of 2-mercaptoacetate is so far unknown. Of interest is that increased amounts of this compound are found in urine from subjects with the rare metabolic disorder 3-mercaptolactate cysteine disulfiduria [2,3], a condition caused by a deficiency of the enzyme 3-mercaptopyruvate sulfurtransferase [3,4]. This results in an accumulation of 3-mercaptopyruvate in the body of affected subjects [3] and it was consequently surmised that 3-mercaptopyruvate is a metabolic precursor to 2-mercaptoacetate.

We report the conversion of 3-mercaptopyruvate to 2-mercaptoacetate by rat mitochondria supplied with NAD^+, CoA and thiamin pyrophosphate (TPP) as cofactors.

2. MATERIALS AND METHODS

Ammonium 3-mercaptopyruvate was synthesized as in [5]. Rat brain homogenates were prepared in 0.44 M sucrose and a crude mitochondrial fraction was prepared according to [6] and its protein content determined as in [7]. The conversion of 3-mercaptopyruvate to 2-mercaptoacetate was studied under reaction conditions similar to those used for the decarboxylation of 4-methylthio-2-oxobutyrate [8] but a lower pH had to be used as we found in preliminary experiments that the spontaneous decarboxylation of 3-mercaptopyruvate drastically increased with an increase of pH. Under the conditions of our assay system the spontaneous formation of 2-mercaptoacetate from 1 μmol of 3-mercaptopyruvate was about 15 nmol and the values presented in tables 1,2 are corrected for this blank reaction. Our standard assay system thus contained 70 μmol potassium phosphate buffer (pH 6.5), 2 μmol magnesium sulfate, 4 μmol NAD^+, 2 μmol CoA, 0.2 μmol TPP and 1 μmol ammonium 3-mercaptopyruvate in a final volume of 2.0 ml. Incubations were performed at 37°C for 30 min with shaking in 25-ml Erlenmeyer flasks. The reaction was terminated by the addition of 40 μl 1 M acetic acid to give pH 5.0 and 0.10 ml of 1.25 M methylhydroxylamine hydrochloride in 2 M sodium acetate and the sample was incubated for 2 h at room temperature. By this step 3-mercaptopyruvate remaining in the assay was converted to the metoxime, which in contrast to the parent compound is stable during gas chromatog-

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raphy [3]. The sample was then deproteinized by the addition of 0.5 ml of 16% trichloroacetic acid followed by centrifugation and a 2-ml aliquot of the supernatant was taken for determination of 2-mercaptoacetate by gas chromatography as in [9].

3. RESULTS AND DISCUSSION

In preliminary experiments we studied the ability of certain rat tissues to form 2-mercaptoacetate when incubated with 3-mercaptopyruvate in phosphate-buffered saline. Of the tissue preparations studied (diaphragm and slices of liver, kidney and brain) only brain produced significant amounts of 2-mercaptoacetate. Further experiments showed that brain homogenates also could form 2-mercaptoacetate and this reaction was strongly inhibited by the dithiol reagent phenylarsine oxide but not by the monothiol reagent *p*-chloromercuribenzoate (table 1). This suggested the participation of a lipoate-dependent decarboxylase analogous to other 2-oxoacid decarboxylases [10]. It may then be presumed that the decarboxylation of 3-mercaptopyruvate to 2-mercaptoacetate required NAD^+, CoA and TPP as cofactors and that the reaction occurred in the mitochondria. In fact, brain mitochondria were found to convert 3-mercaptopyruvate to 2-mercaptoacetate but this reaction was only moderately stimulated by these cofactors when intact mitochondria were used (table 2). However, when the mitochondrial structure was disrupted by freezing and thawing in the presence of the detergent Triton-X-100 [8] an absolute requirement for NAD^+ and CoA and a moderate stimulation by TPP could be demonstrated (table 2). It should be noted that the treatment used for disruption of mitochondria destroyed about 50% of their 3-mercaptopyruvate decarboxylating activity. Of interest is that the expected product of this decarboxylase is 2-mercaptoacetyl CoA and not 2-mercaptoacetate. Unfortunately, the method used for the determination of 2-mercaptoacetate [9] required a step in which the sample is incubated at pH 10 with thiopropyl–Sepharose, which can be expected to convert 2-mercaptoacetyl CoA to 2-mercaptoacetate [11]. However, further experiments indicated that the ultimate product formed by the action of brain mitochondria on 3-mercaptopyruvate was 2-mercaptoacetate and not its CoA ester. Thus the product, similar to mercaptoacetate, could be completely extracted from the acidified sample with ethyl acetate, whereas short chain acyl esters of CoA are not extracted by this procedure. Furthermore, reduction of the assay mixture with borohydride prior to determination of mercaptoacetate, a treatment which should convert 2-mercaptoacetyl CoA (but not 2-mercaptoacetate) to 2-mercaptoethanol [12], did not decrease the yield of mercaptoacetate and no 2-mercaptoethanol was detected by gas chromatography. Nevertheless, we suggest that the

Table 1

Formation of 2-mercaptoacetate from 3-mercaptopyruvate with brain homogenate and its inhibitor with thiol reagents

Inhibitor[a]	2-Mercaptoacetate formed (nmol)
None	42
Phenylarsine oxide	0
p-Chloromercuribenzoate	37

[a] The assay contained 1 ml of a 10% brain homogenate and inhibitors at a final concentration of 0.1 mM

Table 2

Cofactor requirements for 3-mercaptopyruvate decarboxylation with brain mitochondria

Mitochondria	Cofactors	2-Mercaptoacetate formed (nmol)
Intact[a]	Complete	64
Intact	None	41
Disrupted[b]	Complete	36
Disrupted	$-NAD^+$	0
Disrupted	−CoA	0
Disrupted	−TPP	26

[a] The assay contained 3.0 mg mitochondrial protein

[b] The assay contained 3.8 mg mitochondrial protein from a mitochondrial preparation frozen and thawed 3 times after the addition of Triton X-100 to a final concentration of 1%. The same amount of the original mitochondrial preparation formed 74 nmol 2-mercaptoacetate in the presence of NAD^+, CoA and TPP

primary product formed by oxidative decarboxylation of 3-mercaptopyruvate is 2-mercaptoacetyl CoA, but the latter compound is rapidly hydrolyzed by the short chain acyl CoA hydrolase abundantly present in rat brain mitochondria [13]. Note that evidence has recently been presented [14] for the formation of 2-mercaptoacetyl CoA from 2-mercaptoacetate, CoA and ATP by rat liver mitochondria or purified acetyl CoA synthase. The interesting possibility that 2-mercaptoacetyl CoA may participate in the biosyntheses of other sulfur compounds should also be considered.

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