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Short communication

Rhodanese (thiosulfate:cyanide sulfurtransferase) from frog *Rana temporaria*

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Abstract

The molecular mass of rhodanese from the mitochondrial fraction of frog *Rana temporaria* liver, equaling 8.7 kDa, was determined by high-performance size exclusion chromatography (HP-SEC). The considerable difference in molecular weight and the lack of common antigenic determinants between frog liver rhodanese and bovine rhodanese suggest the occurrence of different forms of this sulfurtransferase in the liver of these animals.

Keywords: Rhodanese; Thiosulfate:cyanide sulfurtransferase

1. Introduction

Thiosulfate:cyanide sulfurtransferase (rhodanese, EC 2.8.1.1) has been found in the liver of a variety of vertebrate species [1]. In mammals it is present solely in the mitochondria of the liver cells [2]. However, in frog *Rana temporaria*, rhodanese activity was detected in both the mitochondria and cytosol [1]. Rhodanese is involved in L-cysteine desulfuration that is important as a source of metabolically active reduced sulfur [3]. The enzyme exhibits particular affinity towards certain sulfur donors of either inorganic (e.g. thiosulfate) or organic (e.g. polysulfides, such as thiocystine, persulfides, such as thiocysteine) origin and effects transfer of a sulfur atom to various nucleophilic acceptors via an enzyme-sulfane ''transition state''. Thus, it participates in cyanide detoxification [5], FeS clusters formation [6] or enzymatic activity regulation [4] (Scheme 1). Recently, the inhibitory effect of trisulfides, containing a bound sulfur, in biological peroxidation systems has been announced [7].

The aim of the present studies was to estimate molecular weight of the mitochondrial rhodanese from frog liver, and to investigate the cross-reactivity between this enzyme and bovine rhodanese. Bovine liver rhodanese, by far more frequently studied variant, is a monomeric protein of approximately 32 kDa [8].

2. Experimental

2.1. Materials

Mature male frogs (*Rana temporaria*) were collected from their wintering places in the country around Cracow.

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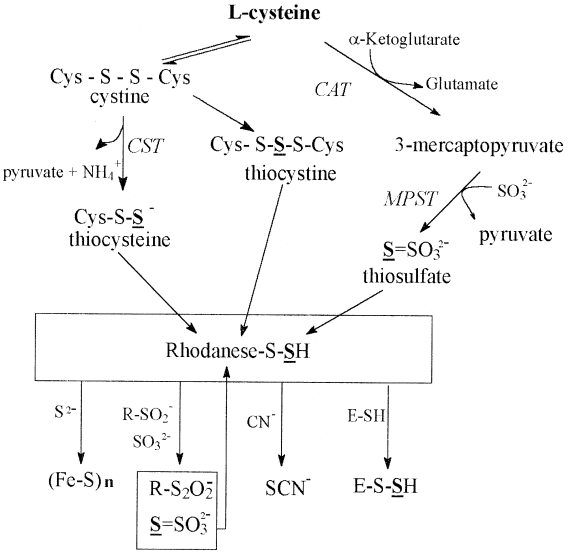

S - sulfane sulfur
CAT - cysteine aminotransferase
MPST - 3-mercaptopyruvate sulfurtransferase
CST - γ-cystathionase

Scheme 1. The participation of rhodanese in L-cysteine desulfuration.

Bovine rhodanese and reagents were obtained from Serva (Heidelberg, Germany), Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany) and were of the highest purity available. Cibacron Blue F3Ga dye was purchased from Fluka (Buchs, Switzerland) and Amicon YM3 membranes from Amicon, Inc. (Beverly, MA, USA).

2.2. Purification procedure

Frogs were decapitated and the spinal cord pithed. The livers were excised, washed with cold 0.9% sodium chloride solution, homogenized in five volumes of a solution containing 250 m*M* sucrose, 5 m*M* EDTA-Na2 (2-low index) and 10 m*M* Tris, final pH 7.4, using a Teflon–glass homogenizer, and the mitochondrial fraction was obtained as described earlier [1].

Frog rhodanese was isolated from the mitochondrial fraction of liver homogenate using the selective binding of the enzyme to agarose immobilized Cibacron Blue F3GA and subsequent elution with the substrate (i.e. thiosulfate) according to the method described by Horowitz [9].

2.3. Size-exclusion chromatography

This was performed on a TSK G2000 SW column (300×7.5 mm, 125 Å pore diameter) using the KONTRON HPLC system. Samples containing 7–14 μg protein were injected into the column. Elution (0.5 ml/min) was carried out with 0.1 *M* phosphate buffer (pH 7.2) containing 0.3 *M* sodium chloride. The protein effluent was monitored at 220 nm.

Aprotinin (6.5 kDa), cytochrome C (12.4 kDa), bovine serum albumin (66 kDa) and the bovine rhodanese (33 kDa) were used as standard proteins for column calibration.

To determine the enzyme activity according to the method of Sörbo [10], fractions of eluent (200 μl) were collected in test tubes containing 400 μl of 0.125 *M* sodium thiosulfate and 200 μl 0.2 *M* potassium phosphate. Then, 200 μl of 0.25 *M* sodium cyanide was added and incubation was performed during 1 h at room temperature, after which thiocyanate was estimated colorimetrically at 460 nm after the addition 0.2 ml 38% formaldehyde and 1 ml ferric nitrate reagent.

The fractions containing rhodanese activity were pooled and concentrated by Amicon YM3.

2.4. Immunoprecipitation and immunodiffusion

Antisera against mitochondrial frog rhodanese and bovine rhodanese were raised in rabbits by multipoint injection of a sample containing 250 μg of protein. Sera showing the strongest reactivity against appropriate antigens were directly used for crossed immunodiffusion or quantitative immunoprecipitation.

The enzyme solution containing about 10 μg of protein in a volume of 10 μl was mixed with either 10 μl of rabbit anti-mitochondrial frog rhodanese or 2–5 μl of rabbit anti-bovine rhodanese or with 2–20 μl of control serum. Each sample mixture was first left at room temperature for 30 min and then for at least 20 h at 4°C. Protein A-bacterial adsorbent pellet prepared from 80 to 160 μl of protein A suspension (10%, w/v) was added to the above mixture and the

sample was kept on ice for 60 min with intermittent shaking. The mixtures were centrifuged for 5 min at 11 530 *g* at 4°C. The supernatants were used for determination of the rhodanese activity.

Immunodiffusion in 1% agarose gel was carried out by the method of Ouchterlony [11] and protein content was determined by the method of Lowry et al. [12] using crystalline bovine serum albumin as a standard.

3. Results and discussion

Rhodanese prepared from the mitochondrial fraction of frog *Rana temporaria* liver according to Horowitz [9] was partially purified 20-fold with a yield of 1% by column chromatography on Cibacron Blue Sepharose. The resulting enzyme preparation had a specific activity of 18.5 U/mg protein, determined according to Sörbo [10], using thiosulfate as a substrate.

The subsequent size-exclusion chromatography yielded one peak of rhodanese activity (Fig. 1), with molecular weight of 8.7 kDa (Figs. 2 and 3). Thus our results are in satisfactory agreement with the previously reported value 9 kDa, obtained by Sephadex G-200 thin-layer gel filtration of the partially purified rhodanese from the mitochondrial fraction of the frog *R. temporaria* liver [13]. Bovine liver rhodanese is a monomeric protein of approximately 32 kDa [8]. A substantial similarity has been

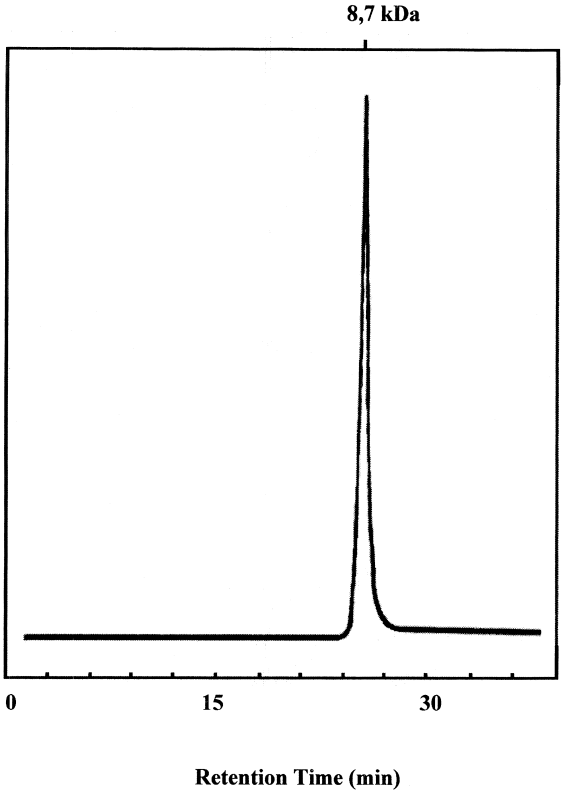

Fig. 1. Chromatographic profile of mitochondrial frog rhodanese after purification on Cibacron Blue sepharose.

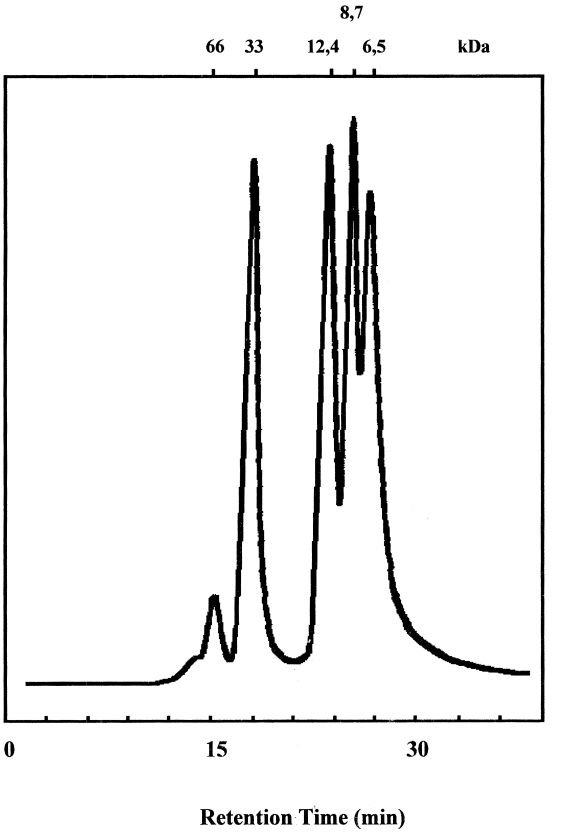

Fig. 2. Chromatographic profile of mitochondrial frog rhodanese (8.7 kDa) and standard proteins: aprotinin (6.5 kDa), cytochrome C (12.4 kDa), bovine serum albumin (dimer 66 kDa, and monomer 33 kDa). Column: TSK G2000 SW (7.5×300 mm). Eluent: 0.1 *M* phosphate buffer (pH 7.2) containning 0.3 *M* sodium chloride. Flow rate: 0.5 ml/min. Absorbance at 220 nm. Retention times: bovine serum albumin (dimer 15.7 min, and monomer 18.3 min), cytochrome C (24.3 min), frog rhodanese (26 min), aprotinin (27.3 min).

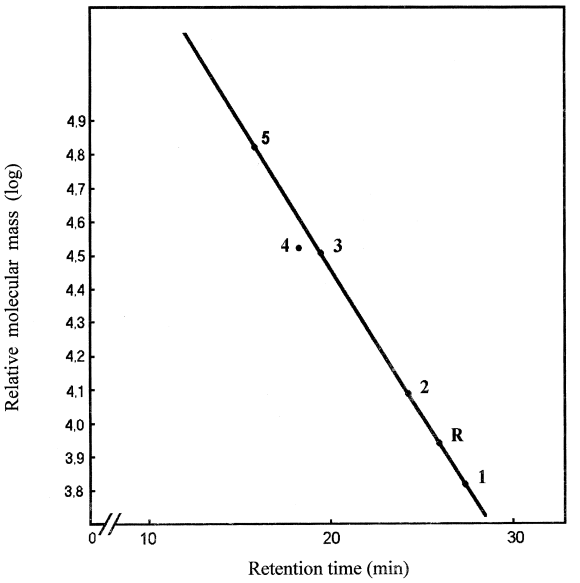

Fig. 3. Calibration of the chromatographic column. Retention time (min) versus relative molecular mass of protein; (1) aprotinin – 27.3 min; (2) cytochrome *c* – 24.3 min; (R) frog rhodanese – 26 min; (3) bovine rhodanese – 19 min; (4) bovine serum albumin (monomer) – 18.3 min; (5) bovine serum albumin (dimer) – 15.7 min.

found for rhodanese sequences from various sources. These include chemically derived protein sequences for avian [14] and bovine liver [8], as well as sequences deduced from the cDNAs from rat [15], mouse [16] and human liver [17]. In contrast to the findings with *Rana temporaria* Sephadex G-75 gel filtration of the partially purified enzyme from the flagellate Euglena gracilis gave a single peak of activity with molecular weight of approx. 27 kDa [18].

The cross-reactivity between frog rhodanese isolated from mitochondrial fractions of liver and beef rhodanese was tested using both the method of immunodiffusion in 1% agarose, as well as the immunoprecipitation with protein A from Staphylococus aureus. No cross-reactivity was demonstrated using either rabbit antiserum against beef enzyme or rabbit antiserum against frog enzyme. These results are in agreement with a previous report; it was found that immunodiffusion of the antiserum to bovine rhodanese against liver extract from different animals shows a partial cross reactivity only within the group of mammalian species [19].

In conclusion, the large difference in molecular weight and the lack of common antigenic determinants suggest the occurrence of different forms of this mitochondrial rhodanese in beef and frog liver.

Acknowledgements

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