Acta Neurobiol. Exp. 2001, 61: 69-72

Sulfurtransferase activity and sulfur compound content in *Rana temporaria* brain following hibernation

Maria Wróbel

Institute of Medical Biochemistry, Jagiellonian University, Collegium Medicum, 31-034 Cracow, 7 Kopernik St., Poland, Email: mbwrobel@cyf-kr.edu.pl

Abstract. The activity of rhodanese, 3-mercaptopyruvate sulfurtransferase and γ-cystathionase and the content of glutathione and sulfane sulfur compounds were determined in *Rana temporaria* brain in April. The high sulfane sulfur level observed in the spring seems to be associated with protection against cellular oxidative stress after the period of hibernation with its minimal oxidative metabolism.

Key words: sulfane sulfur, sulfurtransferases, glutathione, frog brain

In the brain of the frog *Rana temporaria* the pathway of L-cysteine desulfuration providing sulfane sulfur-containing compounds *via* the γ-cystathionase (CST, EC 4.4.1.1) reaction seems to be of greater importance than that *via* the 3-mercaptopyruvate sulfurtransferase (MPST, EC 2.8.1.2) reaction (Wróbel et al. 2000) (Scheme 1). The MPST activity in frog brain was found to be low in comparison to that in rat, while the CST activity was many times higher (Wróbel et al. 1997). γ-Cystathionase and MPST catalyze reactions resulting in the generation of endogenous reduced sulfur while rhodanese (thiosulfate sulfurtransferase, EC 2.8.1.1) carries a sulfane sulfur atom from a variety of sulfur donors (Wood 1982) to various acceptors, for example to cyanide for its detoxification (Westley 1980) and to proteins for iron-sulfur clusters formation (Finazzi-Agro 1971) or to enzymes for their activity regulation (Toohey 1989, Ogasawara et al. 1997).

L-Cysteine desulfuration in *Rana temporaria* brain in the course of the fall migration to wintering sites and the period of hibernation was characterized previously (Wróbel et al. 2000). The changes observed in the activity of the studied enzymes and sulfane sulfur compound levels seem to be correlated in the brain with a diminished mitochondria-related oxidative metabolism during the period of hibernation. The significantly diminished level of glutathione (GSH) in the brain in January may result from a markedly lower demand for this important natural antioxidant at low oxygen consumption and depressed aerobic processes generating oxygen radical species.

The aim of the present study was to investigate L-cysteine desulfuration in *Rana temporaria* brain in April, the beginning of the mating season characterized by increased thyroid activity and maximal oxygen consumption, in order to compare it with that described during the fall migration to wintering sites or during hibernation (Wróbel et al. 2000).

Mature *R. temporaria* male were collected from their wintering places in the country around Cracow in April. Frogs were decapitated and the spinal cord pithed. Brains were excised, washed with cold saline, homogenized in five volumes of 50 mM potassium phosphate buffer containing 1mM EDTA using a Potter-Elvehjem homogenizer with a Teflon pestle. The MPST activity was assayed according to the method of Valentine and Frankenfeld (1974), rhodanese according to Sörbo (1955), γ-cystathionase according to Matsuo and Greenberg (1958) following procedures described earlier (Wróbel et al. 1997). Sulfane sulfur was determined by the method of Wood (1987) based on cold cyanolysis and colorimetric detection of ferric thiocyanate complex ion. Determinations of GSH were performed according to Tietze (1969). Sigma Chemical Company (Deisenhofen, Germany) provided sodium sulfite, N-ethylmaleimide, dithiothreitol, NADH, NADPH, glutathione reductase, 5,5'-dithiobis-(2-nitrobenzoic acid), homoserine, pyridoxal phosphate, α-ketobutyrate, 2-mercaptoethanol and lactate dehydrogenase (EC 1.1.1.27) from pig heart. Sodium thiosulfate and potassium cyanide were obtained from E. Merck, Darmstadt, Germany. Ammonium 3-mercaptopyruvate was synthesized according to Kun (1957).

The results were expressed as the means ±SD (standard deviation) obtained from five frogs.

The MPST activity determined in April (Table I) was approximately five times higher in comparison with that found in October (Wróbel et al. 2000), whereas the value of rhodanese activity was two times higher, and the content of sulfane sulfur three times higher. A diminished mitochondria-related oxidative metabolism during the period of hibernation was accompanied in the brain of the frog by a strongly diminished level of GSH and sulfane sulfur compounds (Wróbel et al. 2000). After the drastic winter fall, the level of GSH reached in April a value similar to that of October. Early in the spring the need for a markedly higher level of GSH as an important antioxidant seems to be obvious. At high oxygen consumption, increased aerobic processes generate radical species and this is accompanied by a significantly in-

Table I

Enzymes activity and metabolites concentration in *Rana temporaria* brain in April

Enzyme activities (μmoles of product/g • min) Metabolite concentrations (μmoles/g fresh weight)	
γ-Cystathionase	0.300 ± 0.020
3-Mercaptopyruvate sulfurtransferase	53.5 ± 0.2
Rhodanese	27.3 ± 1.7
Sulfane sulfur	2.18 ± 0.27
Glutathione	0.483 ± 0.09

The activities of MPST, rhodanese and CST were expressed as μmoles of pyruvate, SCN^- and -ketobutyrate, respectively, per min at 37°C, 20°C and 37°C, respectively, per g of fresh tissue.

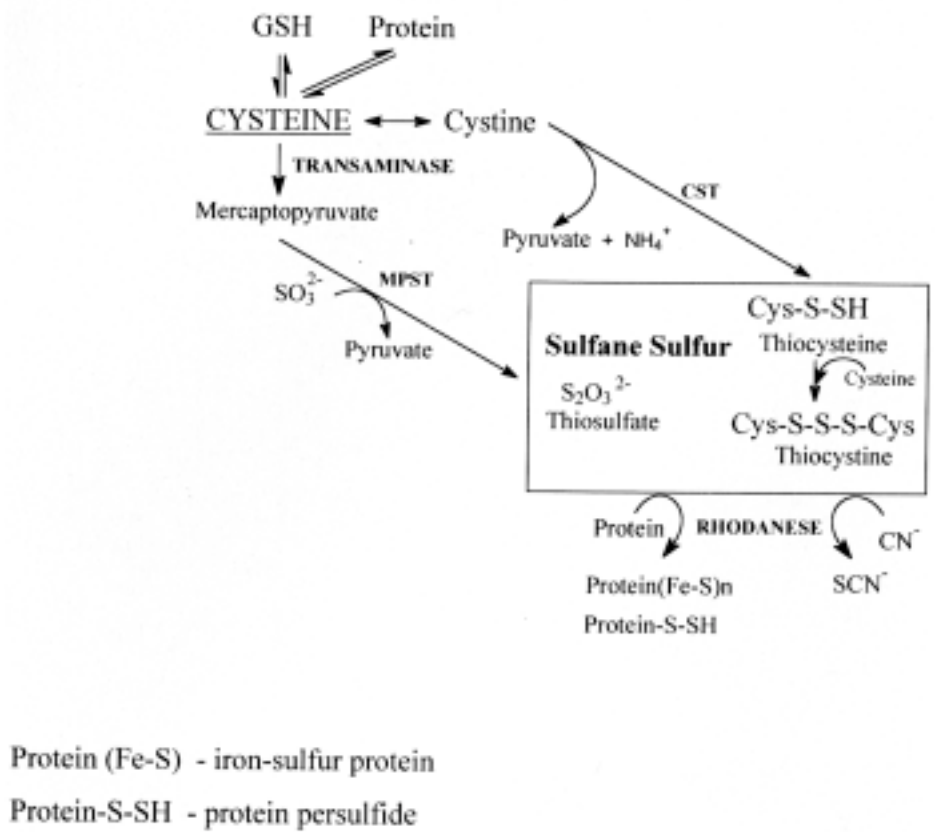

Fig. 1. L-Cysteine desulfuration.

creased level of sulfane sulfur compounds when compared to the period of hibernation (Wróbel et al. 2000). Simultaneously, a much higher level of MPST and rhodanese noted in April indicate that the metabolism of sulfane sulfur compounds is strongly stimulated. It has been found (Ogasawara et al. 1998, 1999) that albumin-bound sulfur and thiocystine (Fig. 1.), typical sulfane sulfur containing compounds, show an antioxidant potential due to the inhibition of cytochrome P-450 which plays an essential role in lipid peroxidation and the generation of radical species. Srebro and Lach (1972) noticed the increased number of cysteine-rich glial cells in the brains of rats and mice after X- and UV-irradiation. They also found (Srebro et al. 1996) such sulfur rich-granules in hepatocytes of frog, near blood vessels, which may serve a detoxifying function. A similar function may be played by granules in mammalians brain.

In conclusion, the changes observed in the brain of *R. temporaria* in rhodanese and MPST activity and in levels of sulfane sulfur-containing compounds and glutathione in spring seem to be clearly associated in spring with protection against cellular oxidative stress after the period of hibernation with its minimal oxidative metabolism.

This work was supported by a grant from the Polish Committee for Scientific Research (KBN) P/136/L.

Finazzi-Agro A., Cannella C., Graziani M.T., Cavallini, D.A. (1971) A possible role for rhodanese: the formation of "labile" sulfur from thiosulfate. FEBS Lett. 16: 172-174.

Kun E. (1957) The reaction of β-mercaptopyruvate with lactic dehydrogenase of heart muscle. Biochim. Biophys. Acta 25: 135-137.

Matsuo Y., Greenberg D.M. (1958) A crystalline enzyme that cleaves homoserine and cystathionine. J. Biol. Chem. 230: 545-560.

Ogasawara Y., Isoda S., Ishii K., Tanabe S. (1997) Modification of liver cytosol enzyme activities promoted *in vitro* by reduced species generated from cystine with cystathionase. Biochim. Biophys. Acta 1334: 33-43.

Ogasawara Y., Isoda S., Tanabe, S. (1998) A labile sulfur in trisulfide affects cytochrome P-450 dependent lipid peroxidation in rat liver microsomes. Toxicol. Lett. 99: 191-198.

Ogasawara Y., Isoda S., Tanabe, S. (1999) Antioxidant effect of albumin-bound sulfur in lipid peroxidation of rat liver microsomes. Biol. Pharm. Bull. 22:441-445.

Sörbo B. (1955) Rhodanese. In: Methods in enzymology (Eds. S. P. Colowick and N. O. Kaplan). Vol. 2. Academic Press, New York, p. 334-337.

Srebro Z., Lach H. (1972) X-ray and UV-induced increase in number of cysteine-rich periventricular glial cells in the brains of rats and mice. Acta Biol. Acad. Hung. 23: 145-151.

Srebro Z., Lach H., Sura P. (1996) Gomori-positive and sulphur-rich granules in hepatocytes of various species of frogs. Acta Biol. Cracov. Ser. Zool. 38: 1-3.

Tietze F. (1969) Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Anal. Biochem. 27: 502-522.

Toohey J.L. (1989) Sulphane sulphur in biological systems: a possible regulatory role. Biochem. J. 264: 625-632.

Valentine W.N., Frankenfeld J. K. (1974) 3-Mercaptopyruvate sulfurtransferase (EC 2.8.1.2): A simple assay adapted to human blood cells. Clin. Chim. Acta 51: 205-210.

Westley J. (1980) Rhodanese and the sulfane pool. In: Enzymatic basis of detoxification. New York, Academic Press, p. 245-262.

Wood J.L. (1982) Biochemical functions of persulfides. Adv. Exp. Med. Biol. 148: 327-342.

Wood J.L. (1987) Sulfane sulfur. In: Methods in enzymology (Eds. S. P. Colowick and N. O. Kaplan). Vol. 143. Academic Press, San Diego, p. 25-29.

Wróbel M., Sura P., Srebro Z. (2000) Low-molecular-weight sulfur compounds and their metabolism in tissues of frog Rana temporaria. Comp. Biochem. Physiol. 125B: 211-217.

Wróbel M., Ubuka T., Yao W-B., Abe T. (1997) L-cysteine metabolism in guinea pig and rat tissues. Comp. Biochem. Physiol. 116B: 223-226.

Received 13 July 2000, accepted 22 November 2000