

## Kinetic characteristics of the reaction of natural thiols with peroxy radicals and hydrogen peroxide

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Kinetic characteristics of the reaction between natural thiols, *viz.*, glutathione, cysteine, and homocysteine, and peroxy radicals formed upon decomposition of the azo initiator AAPH (2,2'-azobis(2-methylpropionamide) dihydrochloride) in aqueous solutions at 37 °C were determined by the competing reaction method. The kinetics of radicals formation in the reaction between natural thiols and H<sub>2</sub>O<sub>2</sub> was studied by the inhibitor method. The polymethine dye, pyridinium 3,3'-di- $\gamma$ -sulfopropyl-9-methylthiocarbocyanine, was used as the radical scavenger. Cysteine was found to exhibit the highest activity in the reactions with peroxy radicals and in the free radical generation upon the reaction with H<sub>2</sub>O<sub>2</sub>. The data obtained can be useful for understanding the physiological role of thiols in oxidative processes.

**Key words:** free radical generation, glutathione, cysteine, homocysteine, antioxidants, kinetics, hydrogen peroxide.

Sulfur-containing compounds are well known destructors of peroxides. They are commonly used in antioxidant compositions for stabilization of oils.<sup>1</sup> Natural thiols also act as antioxidants: they react with hydroxyl radicals, reduce hydrogen peroxide, hydroperoxides, and disulfide —S—S— bonds, and prevent oxidation of proteins.<sup>2–5</sup> Low-molecular natural thiols, glutathione (GSH)<sup>6–9</sup> and cysteine (CSH),<sup>10–12</sup> are used as medicines and hyperhomocysteinemia (elevated blood level of homocysteine (HSH), an amino acid differing from cysteine by an additional methylene group) is indicative of the folic acid and vitamin B<sub>12</sub> deficiency in a body and can result in ischemic heart disease, thrombosis, and psychoneurological diseases.<sup>13–17</sup>

In reactions with free radicals, natural thiols form thiyl radicals which catalyze the *cis*–*trans* isomerization of unsaturated >C=C< bonds.<sup>18–20</sup> It was found<sup>21</sup> that mercaptoethanol can accelerate oxidation of hydrocarbons and methyl linoleate due to the reaction with hydroperoxides, primary oxidation products, which produces free radicals in low yield.

In the present work, the kinetics of the reactions of natural thiols (GSH, CSH, and HSH) with peroxy radicals formed upon decomposition of AAPH (2,2'-azo-

bis(2-methylpropionamide) dihydrochloride) in aqueous solutions, as well as with hydrogen peroxide were studied. Special attention was given to determination of the yield of radicals in the reaction between thiols and H<sub>2</sub>O<sub>2</sub>.

### Experimental

Thiols GSH, HSH, and CSH (Sigma-Aldrich), hydrogen peroxide (Usol'ekhimprom), and azo initiator AAPH (2,2'-azobis(2-methylpropionamide) dihydrochloride, Fluka) were used as received. The solvent was distilled water. The concentrations of H<sub>2</sub>O<sub>2</sub> and thiols were controlled by iodometry. The rate of radical formation in the reaction between the thiol and H<sub>2</sub>O<sub>2</sub> was determined by the inhibitor method. The free radical scavenger was the polymethine dye A (pyridinium salt of 3,3'-di- $\gamma$ -sulfopropyl-9-methylthiocarbocyanine betaine) whose concentration was recorded by spectrophotometry (in aqueous solutions<sup>22</sup>  $\epsilon = 0.77 \cdot 10^5$  L mol<sup>-1</sup> cm<sup>-1</sup> at  $\lambda_{\max} = 546$  nm). All reactions were performed at the physiological temperature of 37 °C directly in a temperature-controlled cell (*l* = 1 cm) of Ultraspec 1100 Pro and Shimadzu 3101 spectrophotometers (Center for Shared Use "New Materials and Technologies" at the Institute of Biochemical Physics of the Russian Academy of Sciences).

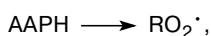
The determination error of kinetic characteristics of the reactions between thiols and peroxy radicals, as well as hydrogen peroxide is not higher than 10%.

## Results and Discussion

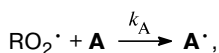
### Antiradical activity of natural thiols

During the detailed study of spectral-kinetic characteristics of several polymethine dyes,<sup>22</sup> it was found that the anionic dye **A** is an efficient scavenger of peroxy radicals and inert to hydroperoxides and hydrogen peroxide. The rate constant of the reaction between dye **A** and peroxy radicals formed upon decomposition of AAPH in water at 37 °C was determined in Ref. 22 to be  $k_A = 5.4 \cdot 10^4 \text{ L mol}^{-1} \text{ s}^{-1}$ , the stoichiometric coefficient being  $f = 1$ . It was found that the anionic dye **A**, in contrast to other polymethine dyes with bulky side substituents,<sup>22</sup> does not react with thiols. This allows one to use this compound for estimation of the antiradical activity of natural thiols by the competing reaction method. Figure 1 demonstrates by the example of glutathione that, at constant rate of radical initiation, the rate of dye consumption decreases upon addition of the thiol, i.e. glutathione acts as a competing radical scavenger.

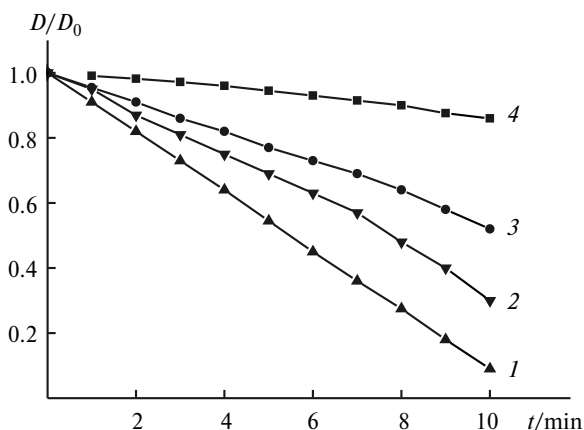
The kinetic scheme for consumption of two competing radical scavengers **A** and **X** (thiols) includes the following reactions:



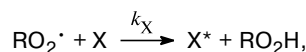
$W_i = k_i [\text{AAPH}]$  is the rate of initiation;



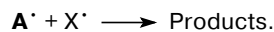
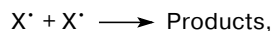
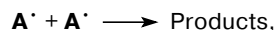
$W_A$  is the rate of consumption of **A**;



**Fig. 1.** Effect of the GSH addition on the rate of dye **A** consumption initiated by AAPH (15 mmol L<sup>-1</sup>);  $[\text{A}] = 0.01 \text{ mmol L}^{-1}$ ,  $[\text{GSH}]$ , mmol L<sup>-1</sup>: (1) 0; (2) 0.004; (3) 0.01; and (4) 0.04.



$W_X$  is the rate of consumption of **X**;



The last three reactions of recombination/disproportionation of radicals **A**<sup>•</sup> formed from the dye and recombination of thiol radicals **X**<sup>•</sup> formed from thiols proceed with high rates<sup>22,23</sup> and provide stoichiometric coefficients  $f = 1$  for dye **A** and thiols in the reactions with peroxy radicals. Under steady-state conditions (at sufficient concentrations of the scavenger), the equation  $W_i = W_A + W_X$  is true, from which it follows that

$$[\text{RO}_2^\cdot] = W_i / \{k_A[\text{A}] + k_X[\text{X}]\}.$$

The rate of dye consumption upon addition of **X** is equal to

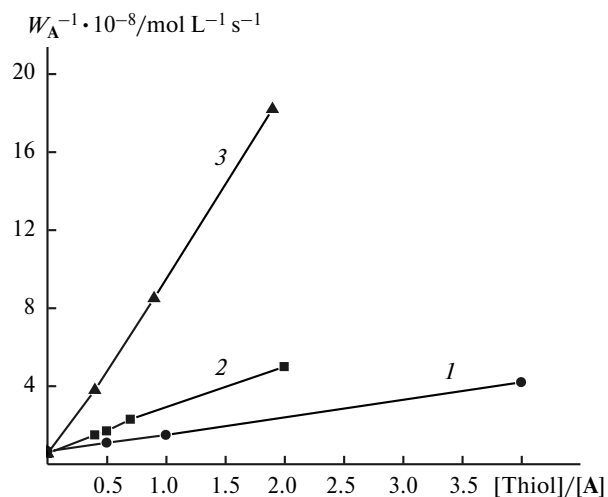
$$W_A = k_A[\text{A}][\text{RO}_2^\cdot] = (k_A[\text{A}]W_i) / \{k_A[\text{A}] + k_X[\text{X}]\} \quad (1),$$

where  $W_i = k_i[\text{AAPH}]$ ,  $k_i$  is the rate constant of radical initiation which, taking into account the cellular effect, is equal at 37 °C to  $1 \cdot 10^{-6} \text{ s}^{-1}$ .<sup>24,25</sup>

To analyze the experimental data, it is convenient to transform Eq. (1) into the form (2):

$$1/W_A = [1 + (k_X/k_A) \cdot ([\text{X}]/[\text{A}])] / W_i \quad (2)$$

Table 1 gives experimentally obtained initial rates of dye **A** consumption upon addition of thiols GSH, CSH, and HSH at different concentration ratios of  $[\text{thiol}] : [\text{A}]$ .



**Fig. 2.** Rate of dye **A** consumption ( $W_A$ ) as a function of the GSH (1), HSH (2), and CSH (3) concentrations in the coordinates of Eq. (2).

**Table 1.** Effect of thiols (HSH, GSH, and CSH) on the rate of dye A consumption in the reaction with radicals formed upon decomposition of AAPH

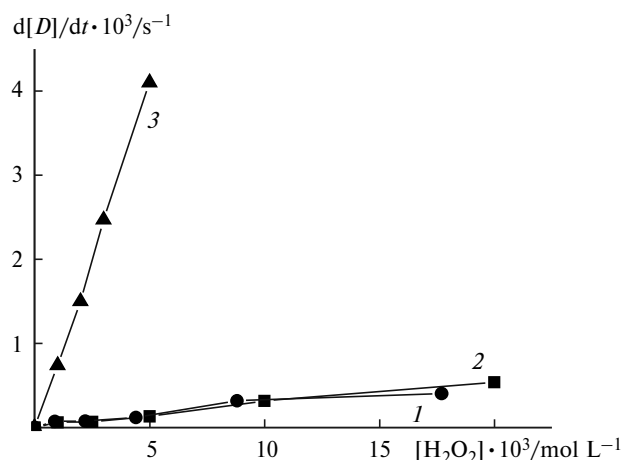
Entry	[Thiol] · 10 <sup>5</sup> /mol L <sup>-1</sup>	[A] · 10 <sup>5</sup> /mol L <sup>-1</sup>	W <sub>A</sub> · 10 <sup>8</sup> /mol L <sup>-1</sup> s <sup>-1</sup>
HSH, [AAPH] = 18 mmol L <sup>-1</sup>			
1	0	1.0	1.8
2	0.25	0.6	0.8
3	0.4	0.66	0.41
4	0.5	0.93	0.62
5	1.0	0.51	0.19
GSH, [AAPH] = 15 mmol L <sup>-1</sup>			
5	0	1.0	1.5
6	0.1	1.0	1.4
7	0.4	0.85	0.97
8	1.0	0.9	0.7
9	4.0	1.0	0.23
CSH, [AAPH] = 18 mmol L <sup>-1</sup>			
10	0	1.0	1.8
11	0.1	0.3	0.2
12	0.2	0.25	0.12
13	0.3	0.2	0.07
14	0.4	0.22	0.055

These data given in the coordinates of Eq. (2)  $1/W_A - [X]/[A]$  fall on straight lines (Fig. 2) whose slope, according to Eq. (2), is equal to  $\text{tg}\varphi = k_X/(k_A \cdot W_i)$ . At known values of  $k_A = 5.4 \cdot 10^4 \text{ L mol}^{-1} \text{ s}^{-1}$  and  $W_i$ ,  $k_X = \text{tg}\varphi \cdot k_A \cdot W_i$ . Table 2 gives the  $k_X$  values which characterize the antiradical activity of thiols as sufficiently strong inhibitors.<sup>26</sup> In a series of thiols, cysteine exhibits the highest activity:  $\text{GSH} < \text{HSH} < \text{CSH}$ .

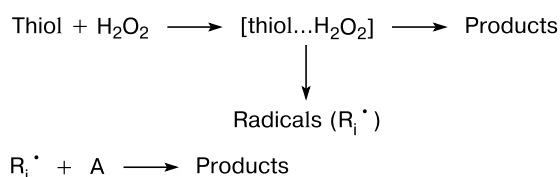
#### **Formation of free radicals in the reactions between thiols and H<sub>2</sub>O<sub>2</sub>**

It is known from Refs 27 and 28 that hydroperoxides and hydrogen peroxide oxidize thiols into disulfides. The decrease in the concentration ratio between the reduced form of the most common thiol, glutathione, and its oxidized form ( $[\text{GSH}]/[\text{GS-SH}]$ ) in cells was considered in many works to be indicative of oxidative stress.<sup>5–10</sup> Special experiments proved that dye A virtually does not react with thiols and hydrogen peroxide. However, upon addition of dye A to the system containing thiol and H<sub>2</sub>O<sub>2</sub> its consumption is observed, which suggests the formation of radicals upon the reaction of peroxide with thiols (Scheme 1).

Obviously, the mechanism of this process includes the initial complexation between thiol and H<sub>2</sub>O<sub>2</sub> followed by transformations of the resulting complex. It was found experimentally that the rate of dye consumption is proportional to the concentrations of H<sub>2</sub>O<sub>2</sub> and thiol (Fig. 3).

**Fig. 3.** Rate of dye A consumption as a function of the H<sub>2</sub>O<sub>2</sub> concentration in the presence of thiols (5 mmol L<sup>-1</sup>): (1) GSH; (2) HSH; and (3) CSH; [A] = 1 · 10<sup>-5</sup> mol L<sup>-1</sup>; aqueous medium, 37 °C.

#### Scheme 1



Therefore, the specific rate of dye A consumption can serve as the kinetic characteristic of free radical formation by thiols in the reaction with H<sub>2</sub>O<sub>2</sub>:

$$\varpi = W_A / \{[\text{H}_2\text{O}_2] \cdot [\text{thiol}]\}.$$

The values of specific rate for thiols GSH, CSH, and HSH are given in Table 2. It is seen from the data given that GSH and HSH almost identically generate radicals and the activity of CSH is 30-fold higher.

The ratio of the rate of glutathione consumption ( $W_{\text{GSH}}$ ) to the rate of radical formation ( $W_A$ ) in the reaction between the thiol and H<sub>2</sub>O<sub>2</sub> is not high to be  $W_A/W_{\text{GSH}} = 0.003$ . However, so low yield of free radicals

**Table 2.** Kinetic characteristics of the reaction between thiols and peroxy radicals ( $k_X$ ) and the radical generation by hydrogen peroxide ( $\varpi$ ) in the aqueous medium at 37 °C

Thiols	$k_X \cdot 10^{-5}$	$\varpi \cdot 10^3$
	L mol <sup>-1</sup> s <sup>-1</sup>	
CSH	4.4	2.1
HSH	2.16	0.07
GSH	0.84	0.07

during the reaction can be enough to initiate chain processes, especially, in multiphase systems.

Thus, by the competing reaction method we have estimated the antiradical activity of glutathione, cysteine, and homocysteine in the reaction with peroxy radicals; their antiradical activities were determined to decrease in the order: CSH > HSH > GSH.

The formation of radicals in the reaction between natural thiols and hydrogen peroxide was found for the first time. The reactivities of thiols in these reactions change in the order: CSH > HSH  $\approx$  GSH. The data obtained may be important for understanding the physiological role of thiols in the overall oxidative process, a source of energy for all living bodies.

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