Oxidation of thiol compounds by molecular oxygen in aqueous solutions

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Side self-oxidation of thiols was studied. It was found that these reactions in neutral and alkaline solutions are induced by impurities of variable-valence metals. The ability of transition metals to catalyze oxidation of thiols changes in the order Cu > Mn > Fe > Ni ≫ Co. The plot of the self-oxidation rate *vs.* pH passes through a maximum whose position on the pH scale depends on both the nature of metal and the structure of the thiol oxidized. For thiols having different structures, the kinetic orders in reactions catalyzed by copper ions differently vary with pH, which is apparently associated with the formation of complexes possessing different catalytic activity.

Key words: thiols, oxidation by molecular oxygen, catalysis by copper ions, effect of the thiol structure on the kinetics.

The reactions of thiol compounds (TC) with molecular oxygen in coordination spheres of variable-valence metals are at the basis of action of many redox metalloproteins, *viz.*, iron-containing proteins (ferredoxin, ferritin, hemerythrin, succinate dehydrogenase), copper-containing proteins (hemocyanin, plastocyanin, ceruloplasmin), *etc.*[1,2] Since reaction mechanisms of these enzymes remain unknown, the development of models of interactions of substrates in the active sites of enzymes is an urgent problem.

The term "self-oxidation of thiols" appeared in the literature in 1950s to mean oxidation of biologically active thiol compounds by oxygen without the addition of variable-valence metal ions.[3] This process is responsible for deterioration of the radioprotective properties of aminothiols[4] (AT) and a decrease in the efficiency of the use of dithiols in the case of poisoning with heavy metal salts.[5]

Thiol compounds in the reduced (RSH) form, *i.e.*, without impurities of their oxidation products, are also required in modern radioelectronic technologies to form monolayer film structures from noble metals.[6]

The data on oxidation of thiol compounds are too contradictory and scarce to estimate the reliability of the proposed reaction mechanisms.[7—11] For example, the dependence of the oxidation rate of bifunctional thiol compounds on pH,[12] inertness of thiols to oxygen in aqueous alkaline solutions, and the fact that aminothiols are easily oxidized in the presence of variable-valence metals[13—16] remain unexplained. The reasons for these contradictions are that reliable analytical methods are lacking, thiol compounds contain large amounts of catalytically active impurities, and data on the effect of the structures of thiols on the characteristic features of their oxidation are unavailable. In the present study, we used analytical methods and procedures for the preparation of high-purity thiol compounds developed by us earlier[17—19] for studying the reactions of these compounds with O_2 under the conditions of both self-oxidation and oxidation in the presence of variable-valence metal ions.

Experimental

Thiol compounds and their oxidation products, including those labeled with the ^{35}S isotope, were synthesized and purified from possible catalytically active impurities according to a procedure described earlier.[19]
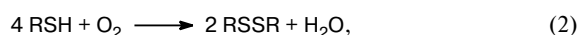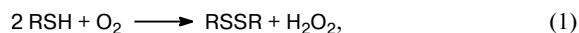
Oxidation products of thiol compounds were analyzed by paper chromatography of reaction mixtures (Watmann-1 paper, 20—25 °C, time of chromatography was 16—18 h). The following solvent systems were used: *A*, EtOH—PrOH—H_2O (40 : 40 : 12); *B*, BuOH—HCOOH—H_2O (75 : 15 : 10); *C*, EtOH—PrOH—1 *M* HCl (40 : 40 : 20); *D*, EtOH—BuOH—HCOOH—H_2O (60 : 20 : 5 : 15); *E*, BuOH—AcOH—H_2O (40 : 10 : 50); and *F*, *n*-C_5H_{11}OH—AcOH—H_2O (45 : 15 : 10). The positions of the reaction products on chromatograms were determined using a developer solution (based on ninhydrin).

In experiments with ^{35}S-labeled thiol compounds, the distributions of radioactivity over chromatograms were determined using an ISOCAPE-300 scintillation detector. The acid ionization constants of SH groups in thiol compounds were determined by spectrophotometry based on UV absorption of thiolate anions (RS^-) in neutral and weak alkaline solutions at $\lambda = 236$ nm at which different thiol compounds have $\varepsilon = (3—5) \cdot 1000$ L mol^{-1} cm^{-1}. For this purpose, equal amounts of
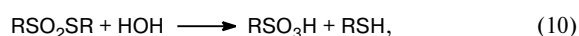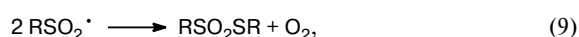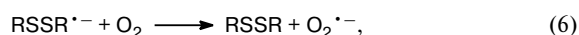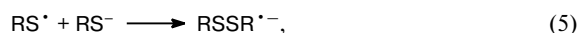
thiol compounds were added to 0.1—0.3 *M* solutions of a borate-phosphate buffer (BPB) in the pH range of 5—12 in 0.25 intervals and then UV spectra of the solutions were recorded. The ratio of the absorbance (*D*) at λ = 236 nm at a particular pH to *D* at pH 12 was taken equal to the percentage of the RS$^-$ form of the thiol compound. The kinetics of oxidation of TC was studied in solutions with pH 4—12 (0.01—0.1 *M* BPB solutions) from which samples were withdrawn. Then the samples were acidified to pH 2—3 and a decrease in the concentration of thiol compounds was determined from the absorption of the thiolate anion form at λ = 236 nm either with the use of Ellman's reagent[20] or based on an increase in the concentration of the disulfide in solutions using UV absorption of an S—S chromophore in acidic (0.1 *M* HCl) solutions at λ = 245 nm and ε = 450 L mol^{-1} cm^{-1}. The concentration of O_2 in kinetic experiments was varied in the range of $5 \cdot 10^{-5}$—$1 \cdot 10^{-3}$ mol L^{-1} by mixing deaerated and oxygen-saturated BPB solutions in required ratios.

Results and Discussion

Self-oxidation of thiol compounds. The reactions of oxygen with thiol compounds in aqueous solutions are most often described by equations

$$2\,RSH + O_2 \longrightarrow RSSR + H_2O_2, \qquad (1)$$

$$4\,RSH + O_2 \longrightarrow 2\,RSSR + H_2O, \qquad (2)$$

according to which the reactions gave disulfides and hydrogen peroxide or water.[11] However, it is known that oxidation of thiol compounds can afford products containing the S atom in different oxidation states depending on the reaction conditions. Under mild conditions (pH 7—9), TC are oxidized by oxygen to disulfides,[15] whereas at pH 12, these compounds are transformed predominantly into sulfinic (RSO_2H) and sulfonic (RSO_3H) acids.[13] Analogous products are also generated upon radiolytic oxidation of aminothiols (Eqs. (3)—(10))

$$RSH \rightleftharpoons RS^- + H^+, \qquad (3)$$

$$RS^\bullet + O_2 \longrightarrow RSO_2^\bullet, \qquad (4)$$

$$RS^\bullet + RS^- \longrightarrow RSSR^{\bullet-}, \qquad (5)$$

$$RSSR^{\bullet-} + O_2 \longrightarrow RSSR + O_2^{\bullet-}, \qquad (6)$$

$$RSH + O_2^{\bullet-} \longrightarrow RS^\bullet + HO_2^-, \qquad (7)$$

$$RS^\bullet + RS^\bullet \longrightarrow RSSR, \qquad (8)$$

$$2\,RSO_2^\bullet \longrightarrow RSO_2SR + O_2, \qquad (9)$$

$$RSO_2SR + HOH \longrightarrow RSO_3H + RSH, \qquad (10)$$

where the RS$^\bullet$ radicals initiate the chain mechanism of oxidation of thiol compounds.[21,22] The possibility of self-oxidation proceeding according to Eqs. (3)—(10) has been demonstrated earlier.[22] In solutions of cysteamine (*C* = 10^{-3} mol L^{-1}) in 0.1 *M* HCl, one-fourth of the starting TC is transformed into the corresponding sulfonic acid

upon storage in air for 4—5 weeks. In neutral and alkaline solutions, the reaction rate increases substantially but thiol compounds are oxidized only to disulfides,[10,11] apparently, according to the scheme described by Eqs. (3)—(8). According to this scheme, either the formation (Eq. (5)) or decomposition (Eq. (6)) of the RSSR$^{\bullet-}$ species is the rate-determining step of the process depending on pH. The kinetics of self-oxidation of TC is indicative of their low reactivity in reactions with O_2. Thus, the second-order rate constants for reactions (1) and (2) in an alkaline medium are in the range of 10^{-4}—10^{-3} L mol^{-1} s^{-1} [13] and are four—five orders of magnitude smaller than the corresponding constants for the reactions of TC, for example, with H_2O_2.[23] This fact is not surprising because the energetics of the transition state of the reaction under consideration is determined by a high barrier (22.5 kcal mol^{-1}) to transition of O_2 from the triplet state to the singlet state in which oxygen acts as the electron acceptor.[24] According to the results of earlier studies,[11,21] the rate of self-oxidation depends linearly on the O_2 concentration. However, the reaction can follow the zero- or first-order kinetics with respect to the TC concentration depending on the source. The bell-shaped plot of the reaction rate *vs.* pH of the medium for oxidation of cysteine (CySH) was attributed[12] to a change of the rate-determining step of the reaction. However, this mechanism is in contradiction with oxidation of other thiol compounds, for example, of cysteamine (ESH), which is characterized by virtually the same pH dependence of the rate, although its radicals ($NH_2CH_2CH_2S^\bullet$) are electroneutral in an alkaline medium. However, if the assumption proposed in the study[12] were true, no decrease in the rate of oxidation of ESH should be observed in the pH range of 9—12.

In addition, the studies[7—11] made different estimates of the degree of reduction of O_2 (from HO$_2$ to H_2O), *i.e.*,

Table 1. Kinetic characteristics of catalytic oxidation of thiol compounds (TC) in the presence of variable-valence metal ions determined according to the equation $W_0 = k[RSH]^x[O_2]^y[M]^z$ (M is metal)

TC	Metal	Degree of reduction of O_2	x	y	z	Reference
Di- and poly-thiols	Fe	H_2O	0, 0.3	0	1	8
H_2S	Co	—	1	1	1	9
H_2S	Cu	HO$_2$	1	1	1	10
Cysteine	Fe	H_2O	0	0	2/3	7
Cysteine	Cu, Fe Mo, Mn	H_2O_2	1	1	2	9
Cysteine	Cu	H_2O_2	−0.5	1	1	11
Cysteine	Cu	H_2O	+0.5	1	1	13
Cysteine	Cu	H_2O_2	0	—	1	14

Table 2. Chromatographic mobilities of thiol compounds

Thiol compounds	Chromatographic system					
	A	B	C	D	E	F
HSCH$_2$CH(SH)CH$_2$SO$_3$Na (unithiol)	0.41	0.69	0.86	0.65	0.58	0.27
CH$_2$CHCH$_2$SO$_3$Na $\vert$ $\vert$ S—S (unithiol disulfide)	0.15	0.46	0.90	0.22	0.35	0.13
Me$_3$N$^+$CH$_2$CH$_2$SH (TSH)*	0.62	0.20	0.50	0.66	0.54	0.25
(Me$_3$N$^+$CH$_2$CH$_2$S)$_2$ (TSST)*	0.28	0.05	0.18	0.50	0.32	0.20
Me$_2$NCH$_2$CH$_2$SH (DSH)	0.46	0.06	0.45	0.62	0.75	0.95
(Me$_2$NCH$_2$CH$_2$S)$_2$ (DSSD)	0.30	0.06	0.25	0.45	0.47	0.43
H$_2$NCH$_2$CH$_2$CH$_2$SH (PSH)	0.66	0.48	0.58	0.75	0.72	0.73
(H$_2$NCH$_2$CH$_2$CH$_2$S)$_2$ (PSSP)	0.27	0.15	0.20	0.55	0.49	0.33
H$_2$NCH$_2$CH$_2$SH (ESH)	0.52	0.45	0.54	0.60	0.53	0.48
(H$_2$NCH$_2$CH$_2$S)$_2$ (ESSE)	0.18	0.15	0.20	0.43	0.20	0.15
H$_2$NCH$_2$CH$_2$SO$_2$H (ESO$_2$H)	—	—	0.42	—	0.37	—
H$_2$NCH$_2$CH$_2$SO$_3$H (ESO$_3$H)	—	—	0.34	—	0.48	—
H$_2$NCH$_2$CH$_2$SSO$_2$CH$_2$CH$_2$NH$_2$	—	—	0.12	—	0.10	—

* Used as perchlorates.

the O$_2$ molecules add from one to four electrons. The data on the kinetic orders with respect to the concentrations of TC (from −0.5 to 1), O$_2$ (from 0 to 1), and variable-valence metals (from 2/3 to 2) are also contradictory (Table 1).

To study oxidation products of thiol compounds, we determined the chromatographic mobilities of TC having different structures, their disulfides, and deeper oxidation products (Table 2). It was demonstrated that disulfides are the only products of transformations of thiol compounds regardless of whether catalytic oxidation is deep (85%) or not (15%). This is evident from a comparison of both a decrease in the concentration of ESH and an increase in the concentration of the corresponding disulfide (ESSE) in the course of self-oxidation at different degrees of oxidation (Table 3).

Since the structure of TC can effect the kinetics both directly (TC can be involved in complexes limiting the reaction rate) and indirectly (by changing the concentration of the reactive thiolate form of TC), we studied acid ionization of SH groups in solutions of thiol compounds having different structures by spectrophotometry in the UV range using absorption of the RS$^-$ chromophore in these compounds.

As can be seen from Table 4, the molar absorption coefficients of thiol compounds with different structures have close values and are in the range of $(3—5.5) \cdot 10^3$ L mol^{-1} cm^{-1}. The constants pK_a^{SH} are in the range of 7.9—10.5. According to the known mechanism,[12] one would expect that the curve of the self-oxidation rate $W_0 = f$(pH) for thioglycolic acid (TGA) will pass through a maximum at pH 10.5 (in accordance with an increase in

Table 3. Comparison of a decrease in the concentration of cysteamine (Δ[ESH]) and an increase in the concentration of the corresponding disulfide (Δ[ESSE]) in the course of self-oxidation of ESH in an aqueous solution*

τ**/h	$2 \cdot \Delta$[ESSE]$\cdot 10^4$	Δ[ESH]$\cdot 10^4$
	mol L^{-1}	
2	5.70	5.90
2	5.30	5.50
2	5.80	6.10
2	5.40	5.70
5	14.9	14.8
5	14.0	14.5
5	14.0	13.8
5	14.4	14.6
20	35.0	37.0
20	39.2	41.5
20	40.2	40.9
20	39.6	38.5

* Reaction conditions: pH 9.25, a borate-phosphate buffer (bidistillate), [ESH]$_0$ = $4.8 \cdot 10^{-3}$ mol L^{-1}, 20 °C.
** The reaction time (four independent experiments).

the proportion of its thiolate form). In actuality, the maximum rates are attained at pH 6.5. For oxidation of ESH and CySH, these dependences go through a flattened maximum at pH 8.5—9.0 (Fig. 1). At the maxima, the rates are 15—20 times higher than those at pH 4—5. An increase in the starting TC concentration in solution leads to a proportional increase in the reaction rate, which could indicate that the reaction follows the first-order kinetics with respect to the concentration of TC. How-

Table 4. Optical properties and pK_a^{SH} for acid ionization of SH groups of thiol compounds (TC) in aqueous solutions (λ = 235 nm, a borate-phosphate buffer)

TC	Absorbing form	$\varepsilon \cdot 10^{-3}$ /L mol^{-1} cm^{-1}	pK_a^{SH} (± 0.05)
ESH	$H_3N^+CH_2CH_2SH$	0.12, 0.10*	—
ESH	$H_2NCH_2CH_2S^-$	5.50	8.35
ESSE	$(H_3N^+CH_2CH_2S)_2$	0.43*	—
CySH	$H_2NCH(COO^-)CH_2S^-$	4.45	8.50
TSH	$Me_3N^+CH_2CH_2S^-$	5.25	7.90
TGA	$^-OOC-CH_2-S^-$	4.42	10.55
(ap)ESH	$H_2N(CH_2)_3NHCH_2CH_2S^-$	5.60	8.60
(dm)ESH	$H_2NCH_2CMe_2S^-$	4.05	8.30
(et)ESH	$H_2NCH_2CHEtS^-$	3.98	8.35
EtSH	$MeCH_2S^-$	3.15	10.50
TPA	$^-OOC-CH_2CH_2S^-$	4.75	10.30
PSH	$H_2NCH_2CH_2CH_2S^-$	5.05	9.35
BSH	$H_2NCH_2CH_2CH_2CH_2S^-$	5.10	9.60

* At λ = 245 nm.

ever, these dependences (straight lines parallel to the horizontal axis) obtained in experiments with the use of dif-
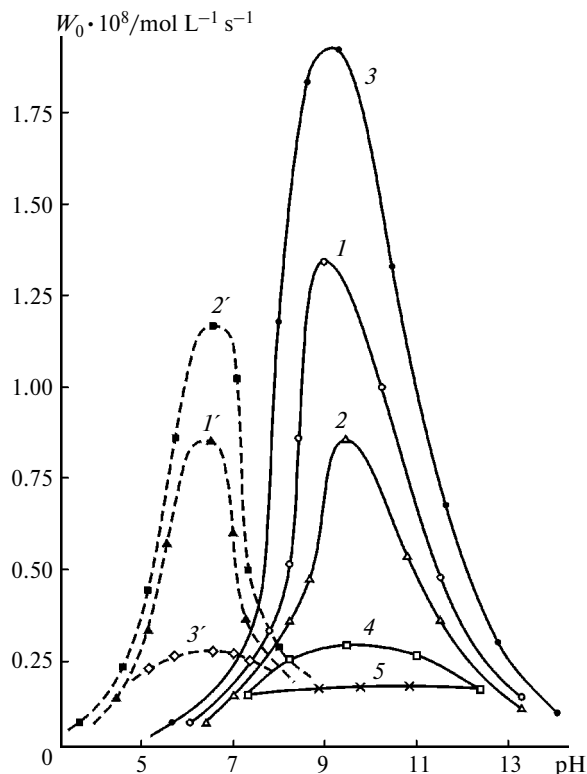
Fig. 1. Dependence of the rates of self-oxidation (W_0) of cysteamine (ESH) (*1—5*) and thioglycolic acid (TGA) (*1´—3´*) on pH of the medium: $[ESH]_0$ = $5.5 \cdot 10^{-4}$ (*1, 2*) and $8.3 \cdot 10^{-4}$ mol L^{-1} (*3—5*), $[TGA]_0$ = $6.2 \cdot 10^{-4}$ (*1´, 3´*) and $8.8 \cdot 10^{-4}$ mol L^{-1} (*2´*), [EDTA] = $5 \cdot 10^{-3}$ mol L^{-1} (*3´, 4*), [KCN] = $5 \cdot 10^{-5}$ mol L^{-1} (*5*); a borate phosphate buffer of special purity (*2*) and analytical grade (*1, 3—5, 1´—3´*).
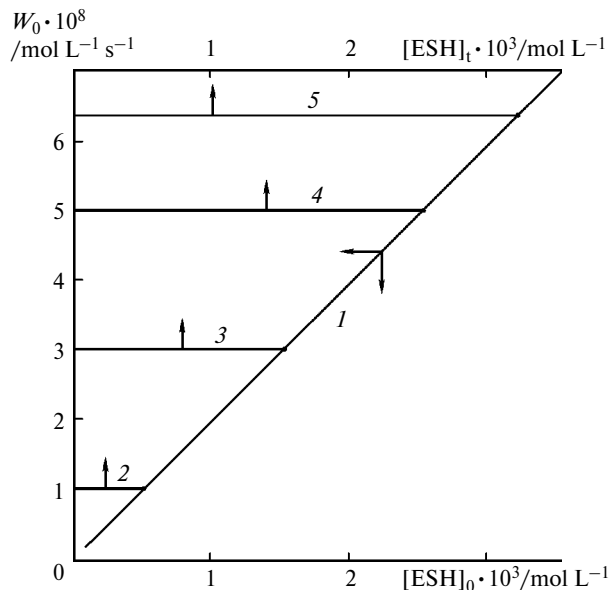
Fig. 2. Dependence of the rates of self-oxidation (W_0) of cysteamine (ESH) on its initial ($[ESH]_0$) (*1*) and running ($[ESH]_t$) (*2—5*) concentrations in aqueous solutions (pH 9.5).

ferent starting concentrations of thiol compounds indicate that self-oxidation follows the zero-order kinetics with respect to TC, *i.e.*, the rate of a decrease in their concentration remains constant until the complete conversion is attained in the reaction with O_2 (Fig. 2). An increase in the concentrations of the components in a borate-phosphate buffer led to an increase in the reaction rate. An analogous effect was observed when buffer solutions were prepared with the use of the reagents of analytical grade instead of those of special purity grade, which should lead to an increase in the concentration of impurities of heavy metals in a 0.05 *M* borate buffer solution from $5 \cdot 10^{-7}$ to $3 \cdot 10^{-6}$ g-ion L^{-1}. The rate of self-oxidation of TC sharply decreases upon the addition of variable-valence metal chelates, such as ethylenediaminetetraacetic acid (EDTA) or cyanide ions (a well-known inhibitor of oxidation of thiolate anions), as well as of acetonitrile, which competes with O_2 for binding to variable-valence metal complexes (see Fig. 1). Finally, the rate of self-oxidation of ESH ($5.1 \cdot 10^{-4}$ mol L^{-1}, pH 9.5, a 0.05 *M* BPB solution) increases by only a factor of 6 as the temperature is increased by 30 °C (from 15 to 45 °C), which is indicative of a low activation energy (~11 kcal mol^{-1}). This activation energy is much lower than that expected for noncatalytic self-oxidation of TC (>20 kcal mol^{-1}) and is more adequate for catalytic processes. Bifunctional thiol compounds containing SH groups along with other functional groups (NH_2, COOH, OH) themselves serve as strong chelating agents for variable-valence metal ions. Apparently, this is the reason why specimens of TC purified on ion-exchange columns[19] were oxidized much more slowly. Therefore, the experi-

mental times of half-oxidation ($\tau_{1/2}$) of thiol compounds (200—500 min) are associated with the presence of catalytically active impurities, *viz.*, variable-valence metal ions introduced into solutions with either components of buffer solutions or TC specimens. Self-oxidation of thiol compounds in itself should proceed with $\tau_{1/2} \geq 10^4$ min and afford oxygen-containing compounds according to the scheme described by Eqs. (3)—(10).[22] In neutral and alkaline solutions, the reaction mechanism changes radically and the observed rather rapid side self-oxidation of thiol compounds according to reactions (3)—(8) can be initiated by catalytically active impurities of variable-valence metals, which generate the RS$^\bullet$ radicals into the bulk of the surrounding solution (the ion-radical mechanism of catalysis). Within the framework of this mechanism, the bell-shaped function $W = f(\text{pH})$, presumably, corresponds to the region of existence of the RSSR$^{\bullet-}$ radical-ionic species, which serve as key participants of chain radical reactions (3)—(8). In this case, either the formation (Eq. (5)) or decomposition (Eq. (6)) of the radicals is the rate-determining step of the reaction depending on pH. However, one cannot rule out an alternative mechanism according to which the reactions of TC with O_2 proceed completely in the coordination sphere of variable-valence metal (the ion-molecular mechanism of catalysis).[24] To elucidate the reaction mechanism, it is necessary to study catalytic oxidation of thiol compounds in the presence of variable-valence metals, investigate the structures of catalytically active species, and reveal the characteristic features of their interactions with the O_2 molecule.

Catalysis of oxidation of thiol compounds by variable-valence metal ions. We studied simple salts of Fe, Cu, Cr, Mn, Ni, Co, and other constructionally important metals instead of known catalytic systems of liquid-phase oxidation, such as phthalocyanines or porphyrins containing variable-valence metals. It is these metals that can be present in commercial products and are actually the major impurities in thiol compounds and other reagents. Hence, they are primarily responsible for instability of TC with respect to O_2 in aqueous solutions. We tested a number of variable-valence metal ions, *viz.*, ions of transition metals and lanthanides, for catalytic activity in oxidation of thiol compounds with the aim of finding out whether the catalytic effect depends on the oxidation state and the ligand environment about the variable-valence metal ion in the starting compound, revealing the influence of the acidity of the reaction medium and the chemical nature of TC subjected to oxidation on the rate of catalytic oxidation, and elucidating whether the kinetic orders of the reaction with respect to the components are retained regardless of pH for oxidation of thiols with different structures.

It was found that the catalytic effect in borate-phosphate buffers (pH 2—13) decreases in the following series
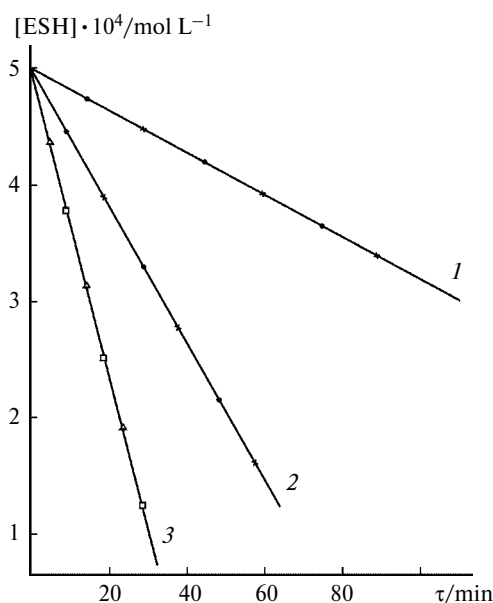

Fig. 3. Oxidation of cysteamine (ESH) in the presence of Cu compounds (CuSO$_4$, CuCl) (*1*, *2*) and Mn compounds (MnSO$_4$, KMnO$_4$) (*3*): *1*, [Cu] = $1.9 \cdot 10^{-7}$ g-ion L^{-1}, pH 9.5, $\tau_{1/2}$ = 140 min; *2*, [Cu] = $2.1 \cdot 10^{-7}$ g-ion L^{-1}, pH 8.0, $\tau_{1/2}$ = 45 min; *3*, [Mn] = $2.0 \cdot 10^{-6}$ g-ion L^{-1}, pH 10.8, $\tau_{1/2}$ = 20 min.

of metals: Cu > Mn > Fe > Ni $\gg$ Co. This effect was demonstrated to be independent of the oxidation state of variable-valence metal in the starting compound, *i.e.*, the following series of compounds exhibit equal activities: MnSO$_4$, MnCl$_2$, and KMnO$_4$; CuSO$_4$, CuCl, and Cu(MeCN)$_4$ClO$_4$; FeSO$_4$ and FeCl$_3$. Any compound in which the metal atom is coordinated by a weak chelating ligand can serve as the catalyst (Fig. 3), whereas strong chelating agents, such as EDTA and cyanide ions, sharply suppress the catalytic activity of the above-mentioned series of variable-valence metals. In acidic media (pH 2—5), thiol compounds are virtually equally resistant to O_2 both in the presence and absence of variable-valence metals in solutions, whereas the oxidation rate in neutral and weak alkaline media (pH 6—12) increases a hundred-fold in the presence of catalytic amounts (10^{-6}—10^{-5} g-ion L^{-1}) of variable-valence metal ions. For all catalytically active variable-valence metals, the rates W_0 pass through a maximum (Fig. 4), the maximum rates of Cu- and Mn-catalyzed oxidation of ESH being observed at pH 8.5 and 10.5, respectively. Hence, it follows that the zwitterionic forms of thiol compounds do not possess specific properties favorable for their oxidation, contrary to popular opinion.[17] Actually, the maximum proportion (0.9) of the zwitterionic form of ESH in equilibrium with other forms is observed at pH 9.5, whereas this proportion is 0.5 and 0.2 at pH 8.5 and pH 10.5 (at the maxima of the rate of oxidation catalyzed by Cu and Mn ions), respectively. It should be noted that the maximum differences in the rates of catalytic oxida-
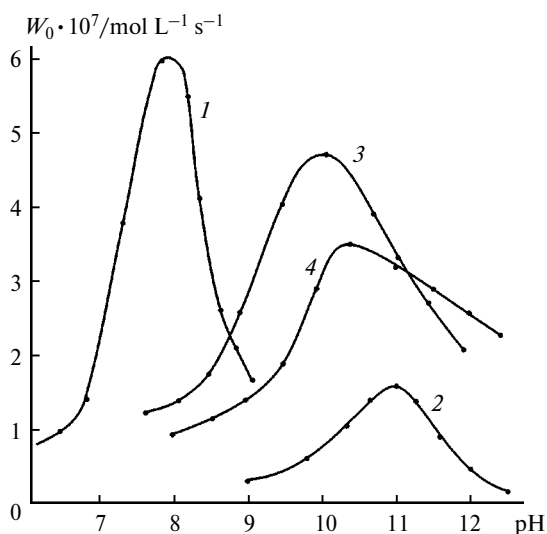
Fig. 4. Dependence of the rates of oxidation (W_0) of cysteamine (ESH) on pH of solutions in the presence of Cu ions (*1*), Mn ions (*2*) ([M] = $5.0 \cdot 10^{-7}$ g-ion L^{-1}), Fe ions (*3*) ([Fe] = $3.2 \cdot 10^{-6}$ g-ion L^{-1}), and Ni ions (*4*) ([Ni] = $1.7 \cdot 10^{-5}$ g-ion L^{-1}) ([ESH]$_0$ = $5.1 \cdot 10^{-4}$ mol L^{-1}).

tion for catalytically active metals are observed at the maxima of the function $W_0 = f(\text{pH})$, and the activity in this series of metals decreases by a factor of ~5 on going from one metal to another under comparable conditions (see Fig. 4). The rate of catalytic oxidation of thiol compounds having different structures catalyzed by the same variable-valence metal also goes through a maximum depending on pH. It should be noted that thiols differ substantially not only in that their maximum oxidation rates are observed at different pH but also in the rates as such (Fig. 5). For example, under comparable conditions, the maximum rates for TGA and PSH are observed at pH 6.2 and 11.3, respectively ($4 \cdot 10^{-6}$ and $5 \cdot 10^{-5}$ mol L^{-1} min^{-1}). The determination of the kinetic orders of catalytic oxidation with respect to the components demonstrated that they are independent of pH but depend on the structure of TC subjected to oxidation. In a neutral medium, the rates are directly proportional to the concentrations of thiol compounds and O$_2$ and depend only slightly on the concentration of variable-valence metal ions. In an alkaline medium, the rates are independent of the concentration of thiol compounds. For some thiol compounds, the rates are also independent of the concentration of O$_2$. Oxidation of particular TC follows either the first- or second-order kinetics with respect to the variable-valence metal ions. In particular, although the structures of ESH and PSH differ insignificantly (PSH contains one CH$_2$ group more than ESH), there are large differences in catalytic oxidation of these thiol compounds in the presence of Cu ions. Thus, oxidation of PSH follows the first-order kinetics with respect to [O$_2$] and [Cu] both in neutral and alkaline media, whereas oxidation of
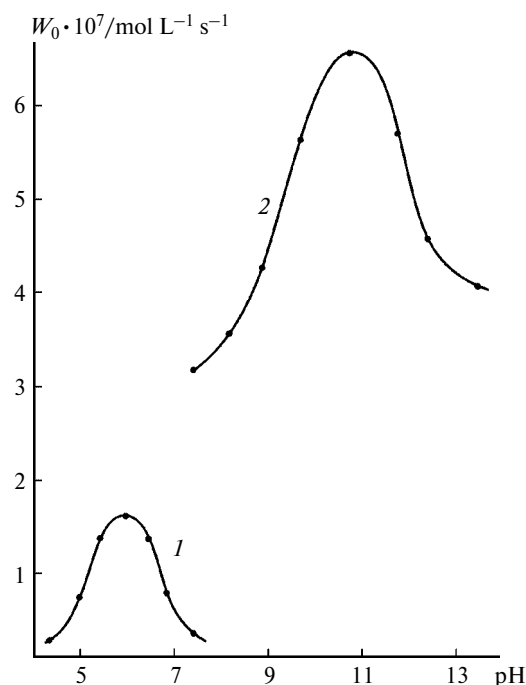

Fig. 5. Dependence of the rates of oxidation (W_0) of thiol compounds (TC) with different structures on pH of solutions in the presence of Cu ions ($1.0 \cdot 10^{-7}$ g-ion L^{-1}) at [TC]$_0$ = $5.0 \cdot 10^{-4}$ mol L^{-1}: TGA (*1*), PSH (*2*).

ESH in an alkaline medium follows the zero-order kinetics with respect to [O$_2$] and the second-order kinetics with respect to [Cu]. These data show that catalytic oxidation of all thiol compounds in a neutral medium proceeds by the same mechanism, whereas oxidation of some thiol compounds (ESH, CySH, *etc.*) in an alkaline medium proceeds according to another mechanism, the mechanism of oxidation of the other thiols (PSH, BSH, TSH) remaining unchanged. The complex kinetic behavior of systems involving TC, variable-valence metal, and O$_2$ in solutions with different acidity and its sensitivity to the chemical structures of the compounds oxidized are apparently associated with the characteristic features of complex formation of thiol compounds with variable-valence metals. Thus, solutions of Cu$^+$ and RS$^-$ as ligands in an alkaline medium in the presence of a large excess of TC with respect to Cu$^+$ contain predominantly the [(Cu$^+$)($^-$SR)$_2$] complexes, which can exist primarily in the linear or chelate form ([(Cu$^+$)($^-$SB)$_2$] or [(Cu$^+$)($^-$SE)$_2$], respectively) depending on the structure of the ligand.[18,19] The above-mentioned characteristic features of catalytic oxidation are, apparently, indicative of substantial differences in the interactions of these forms of complexes with the O$_2$ molecule.

It should be noted that our study revealed drawbacks of conventional procedures, which, in particular, do not allow one to determine the stoichiometry of catalytic oxidation of thiol compounds and study the kinetics of the

reaction in a wide concentration range of reagents. Thus, the monitoring of the process based on a decrease in the concentration of a thiol compound imposes limitations on the latter value, which should be in the range from $5 \cdot 10^{-4}$ to $5 \cdot 10^{-3}$ mol L^{-1} because of low solubility of O_2 in aqueous solutions, whereas the concentration of variable-valence metal ions at high oxidation rates of many thiols can be varied by a factor of no more than 2—3 because of the second order of the reaction with respect to metal. This range in which the concentrations of the reagents can be varied is too narrow to both reliably elucidate the characteristic features of catalytic oxidation and provide conditions for the formation of all kinetically active forms of complexes of thiol compounds with metal ions. In addition, if O_2 is reduced only to H_2O_2 in the course of the reaction, it is not correct to monitor the process based on a decrease in the concentration of TC, because H_2O_2 that formed additionally oxidizes an equivalent amount of the thiol compound and, consequently, only one-half of TC is consumed for catalytic oxidation of interest. Attempts to determine the degree of reduction of O_2 in the course of catalytic oxidation using a known procedure for analysis of H_2O_2 in aqueous solutions[21] failed because of the low sensitivity of this procedure. In rare cases, the presence of H_2O_2 in a system involving TC, variable-valence metal, and O_2 was only detected qualitatively.

To study the stoichiometry and kinetics of catalytic oxidation of thiol compounds, we developed a sensitive method of analysis of H_2O_2 and adapted a known polarographic method of analysis of O_2 to solutions containing aggressive TC, which rapidly poison a measuring electrode. The results of studies with the use of these procedures will be published elsewhere.

References

1. H. Beinert, M. C. Kennedy, and C. D. Stout, *Chem. Rev.*, 1996, **96**, 2335.
2. E. T. Adman, *Adv. Protein Chem.*, 1991, **42**, 145.
3. A. Shoberl, in *Proc. Int. Text. Res. Conf.*, Melburn, 1955, 157.
4. Z. M. Bacq and P. Alexander, *Fundamentals of Radiobiology*, 2nd ed., Pergamon Press, Oxford, 1961, 432 pp.
5. G. I. Oksengendler, *Yady i protivoyadiya* [*Toxins and Antidotes*], Nauka, Leningrad, 1982, 191 pp. (in Russian).
6. G. E. Poirier, *Langmuir*, 1997, **13**, 2019.
7. J. E. Taylor, E. Y. Jonson, and J. L. Wang, *J. Am. Chem. Soc.*, 1966, **88**, 1663.
8. C. G. Overberger, K. H. Burg, and W. H. Daly, *J. Am. Chem. Soc.*, 1965, **87**, 4125.
9. J. Stauff and F. Nummerfoll, *Z. Naturforsch.*, 1969, **24b**, 1009.
10. A. Hanaki and H. Kamide, *Chem. Pharm. Bull.*, 1975, **23**, 1671.
11. D. Cavallini, C. De Marko, and S. Dupre, *Arch. Biochem. Biophys.*, 1968, **124**, 18.
12. R. E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, 1955, **77**, 5877.
13. T. J. Wallace, *Tetrahedron*, 1965, **21**, 2271.
14. A. Hanaki, *Bull. Chem. Soc. Jpn.*, 1995, **68**, 831.
15. L. Pecci, G. Montefoschi, G. Musci, and D. Cavallini, *Amino Acids*, 1997, **13**, 355.
16. A. Kachur, C. J. Koch, and J. E. Biaglow, *Free Radic. Res.*, 1999, **31**, 23.
17. G. A. Bagiyan, S. A. Grachev, I. K. Koroleva, and N. V. Soroka, *Zh. Org. Khim.*, 1975, **11**, 900 [*J. Org. Chem. USSR*, 1975, **11** (Engl. Transl.)].
18. G. A. Bagiyan, S. A. Grachev, and N. V. Soroka, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1975, 435 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 1975, **24** (Engl. Transl.)].
19. G. A. Bagiyan, S. A. Grachev, I. K. Koroleva, and N. V. Soroka, *Zh. Obshch. Khim.*, 1976, **46**, 365 [*J. Gen. Chem. USSR*, 1976, **46** (Engl. Transl.)].
20. G. L. Ellman, *Arch. Biochem. Biophys.*, 1959, **82**, 70.
21. J. P. Barton, J. E. Packer, and R. J. Sims, *J. Chem. Soc., Perkin Trans. 2*, 1973, 1547.
22. S. A. Grachev and N. V. Soroka, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1981, 1756 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 1981, **30** (Engl. Transl.)].
23. G. A. Bagiyan, S. A. Grachev, I. K. Koroleva, and N. V. Soroka, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1976, 990 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 1976, **25** (Engl. Transl.)].
24. Yu. I. Skurlatov, G. G. Duka, D. G. Batyr, and S. O. Travin, *Koord. Khim.*, 1989, **15**, 291 [*Sov. J. Coord. Chem.*, 1989, **15** (Engl. Transl.)].