KINETICS OF THE REACTIONS OF ISOTHIOCYANATES WITH SULPHIDE AND THIOLS

Ľ. Drobnica[a], D. Podhradský[b] and P. Gemeiner[a]

[a] *Department of Microbiology and Biochemistry, Slovak Institute of Technology, 880 37 Bratislava and*
[b] *Department of Organic Chemistry, P. J. Šafárik University, 040 01 Košice*

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The kinetics of the reactions of phenyl isothiocyanate, its 4-substituted derivatives and benzyl isothiocyanate with sodium sulphide, ethanethiol, mercaptoacetic acid, ethyl mercaptoacetate, 2-mercaptoethanol, 2-mercaptopropionic acid, methyl 2-mercaptopropionate, dithiothreitol, and benzenethiol has been studied. All thiol compounds studied react in dissociated form. Under conditions described in this paper isothiocyanates quantitatively react with HS^- or RS^- ion under formation of dithiocarbamates and S-esters of N-substituted dithiocarbamic acids, respectively. Mercaptoacetic acid and its ethyl ester produce 3-substituted rhodanines. The reactivity of phenyl isothiocyanates with HS^- ions and aliphatic thiols obeys the Hammett equation with positive slopes ϱ which vary in a narrow range. The reactivity of thiols increases with their basicity. Comparison of the nucleophilities of OH^- ions, aliphatic alcohols, aliphatic amines, amino acids, HS^- ions, aliphatic thiols and benzenethiol with respect to phenyl isothiocyanate indicates enormous nucleophility of thiols as compared with other nucleophilic agents.

It is known that isothiocyanates (ITC) are successfully used in the chemistry of proteins for instance for the determination of primary structure of proteins and peptides, because they react with amino groups of those substances. On the basis of the preceding pieces of information elucidating the mechanism of antimicrobial or biological activity we have drawn the unambiguous conclusion that the biological activity of ITC* is due to their reactions with SH group of proteins[1]. Therefore, the inhibition of glycolysis in yeasts, bacteria, and animal cells is the result of the inactivation glyceraldehyde-3-phosphate dehydrogenase (GAPDH), hexokinase (HK) as well as other enzymes which require free SH groups for their activity[2,3]. The inhibition of some mitochondrial functions of isolated mitochondria[4,5] as well as

* *Abbreviations*: DTC, N-monosubstituted dithiocarbamic acid or their salt; EDTC, S-ester of N-monosubstituted dithiocarbamic acid; ITC, isothiocyanate; (Nbs_2), 5,5′-dithio-bis-(2-nitrobenzoic acid). Enzymes: GAPDH, Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); glutathione reductase (EC 1.6.4.2); HK, hexokinase (EC 2.7.1.1).

the inhibition of proteosynthesis in a cell free system, *e.g.* inactivation of ribosomes and some elongation factors[6], has been explained by the reaction of SH groups of proteins with ITC. The mechanism of the inactivation of GAPDH and HK enzymes as well as glutathione reductase has been studied in more detail[7,8]. By choosing suitable reaction conditions, especially pH, it is possible according to the reaction with ITC to reveal for above enzymes: *1*) selective reaction with SH groups; *2*) reaction with SSH and NH_2 groups; *3*) the same as *sub. 2*) accompanied by the liberation of SH groups owing to the selective nucleophilic elimination or subsequent controlled acid hydrolysis involving the splitting of N-terminal amino acid with occupied α-amino group.

EXPERIMENTAL

Chemicals. The commercial thiols (Lachema, Reanal, and Koch-Light) were distilled or recrystallized before use. Ethyl mercaptoacetate, b.p. 54·5—55·0°C/17 Torr and methyl 2-mercaptopropionate, b.p. 67·0—68·0°C/20 Torr were prepared by usual esterification method from mercaptoacetic acid or mercaptopropionic acid, respectively. (Nbs_2) was obtained from Aldrich, and benzenethiol was supplied by Dr M. Uher, Department of Organic Chemistry, Slovak Institute of Technology, Bratislava. The concentration of the sulphide and thiols was estimated by spectrophotometric measurements in UV region by using the values of ε taken from literature[9–12], the Ellman method using (Nbs_2) (ref.[13,14]), and iodometric titration. Because of the high volatility of ethanethiol, its concentration was determined immediately before use. ITC were prepared according to Antoš[15] or Dyson[16] and distilled or recrystallized before use. The solvents were spectrally pure, the buffer constituents were analytical grade chemicals. The sodium salt of N-phenyl dithiocarbamic acid, S-carboxyethylester of N-(4-bromophenyl)dithiocarbamic acid, and S-methyl carboxyethylester of N-(4-bromophenyl)dithiocarbamic acid were prepared according to literature[17,18]. The UV spectra of the buffered aqueous solutions of synthetic products were identical with the UV spectra of the reaction mixtures containing corresponding products after the reaction had been completed.

UV Spectra in the 220—360 nm region were recorded with Specord UV VIS spectrophotometer Zeiss (GDR) using 5—30 mm thick cells.

Kinetic measurements were carried out on Specord UV VIS at 25 ± 0·2°C. The buffers were adjusted on pH-meter Radelkis (Hungary) with the accuracy of ±0·01. The reaction mixtures containing at least 20-fold excess of the sulphide or thiol so that all reactions were first order reactions. In most cases the final extinction was constant at least for fivefold half-change period. The initial concentrations of ITC, the sulphide, and thiols were in the range from 25—50 μM, 92—149 μM, and 0·125—1·25 mM, respectively. ITC were dissolved in methanol, the sulphide in distilled water, and thiols either in distilled water or methanol. The resulting mixtures contained 1 vol. % of methanol. The McIlvain 0·2M citrate–phosphate buffer solution (pH 4·5—7·5) and the Sörensen 0·2M borate buffer solution (pH 7·8—11·0) were employed for the preparation of buffered reaction mixtures. The first order rate constants k_{obs} (s^{-1}) were calculated according to Eq. (*1*) and (*2*).

$$k_{obs} = 2{\cdot}3[\log(E_\infty - E_{t_2}) - \log(E_\infty - E_{t_1})]/(t_2 - t_1)\,, \qquad (1)$$

$$k_{obs} = 2{\cdot}3[\log(E_{t_2} - E_0) - \log(E_{t_1} - E_0)]/(t_2 - t_1)\,. \qquad (2)$$

In all cases these relationships were linear at least for two half-change periods. The rate constants k_{obs} were obtained from double kinetic measurements and were reproducible with about 5% deviation from the average value. For the calculation of second order rate constants k ($M^{-1} s^{-1}$)

TABLE I

Rate Constants of the Reaction of Phenyl Isothiocyanate with Sodium Sulphide at Different pH Values

Initial concentration C_6H_5NCS 31·3 μM, Na_2S 920 μM.

pH	$k_{obs} \cdot 10^3$,[a] s^{-1}	c_{HS^-},[b] μM	k $M^{-1}s^{-1}$
7·00	5·39	408	13·20
7·25	7·34	540	13·60
7·50	8·16	658	12·40
7·75	9·67	752	12·87
8·00	10·81	817	13·25
8·25	11·15	858	13·00
8·50	11·29	880	12·82
9·00	11·80	909	13·00
10·00	11·70	919	12·85
11·00	11·80	920	12·82

[a] Apparent rate constant at 25°C; [b] the concentration of HS^- was calculated from Henderson Hasselbach equation.

TABLE II

Rate Constants of the Reactions of 4-Substituted Phenyl Isothiocyanates *I*—*VI* and Benzyl Isothiocyanate (*VII*) with HS^-

Compound	R	λ[a], nm	k_{HS^-},[b] $M^{-1}s^{-1}$	log k_{HS^-}	log k_i/k_0
I	CH_3O	300	7·85 ± 0·24	0·895	−0·225
II	CH_3	295	6·44 ± 0·28	0·809	−0·311
III	H	295	13·18 ± 0·33	1·159	0·000
IV	Br	300	17·90 ± 0·52	1·253	0·133
V	CH_3CO	320	32·90 ± 1·78	1·517	0·397
VI	O_2N	365	59·30 ± 4·13	1·773	0·615
VII	H	285	7·32 ± 0·19	0·865	−0·255

[a] Wavelengths at which the measurements were performed; [b] at 25°C.

the values of k_{obs} measured at those pH values at which the half-change periods were 1·0—1·5 minutes were used. The rate constants k were calculated from the Eq. (*3*):

$$k = k_{obs}(K_a + c_{H^+})/c_0 \cdot K_a , \qquad (3)$$

where K_a (M^{-1}) is the dissociation constant of the sulphide or thiol, c_0 (M) is their initial analytical concentration, and c_{H^+} (M) is the concentration of H^+ ions calculated from the data obtained on pH-meter.

TABLE III

Rate Constants of the Reactions of the 4-Substituted Phenyl Isothiocyanates *I*—*VI* and Benzyl Isothiocyanate (*VII*) with Thiols RSH (25°C)

No[a]	R	k, ($M^{-1}s^{-1}$) for						
		I	*II*	*III*	*IV*	*V*	*VI*	*VII*
2	$H_5C_2OOCCH_2$—	—	103	128	—	—	—	139
3	HSCH(OH)CH(OH)—	89	155	275	220	698	1 091	54
4	$H_3COOCCH_2CH_2$—	985	1 090	1 785	2 245	5 690	—	910
5	$HOCH_2CH_2$—	422	485	607	1 100	3 010	6 500	228
6	$HOOCCH_2$—[b]	—	—	1 900	2 495	4 500	12 050	1 900[c]
7	H_3CCH_2—	394	515	492	773	1 100	—	252
8	$HOOCCH_2CH_2$—	1 760	1 950	2 770	4 580	6 400	—	1 250

[a] No *1* denotes sodium sulphide; [b] kinetic data from ref.[20]; [c] from measurements of the reactivity of 4-bromobenzyl isothiocyanate with respect to mercaptoacetic acid[20].

TABLE IV

Hammett Correlations for the Reactions of the 4-Substituted Phenyl Isothiocyanates *I*—*VI* with Sodium Sulphide and Aliphatic Thiols RSH

No	R[a]	$\varrho_{25°C}$	r[b]	log k_0	n[c]
1	H^+ (Na^+)	0·90 ± 0·09	0·98	1·035	6
3	HSCH(OH)CH(OH)—	0·97 ± 0·07	0·93	2·310	6
4	$H_3COOCCH_2CH_2$—	0·96 ± 0·11	0·98	3·223	5
5	$HOCH_2CH_2$—	1·17 ± 0·08	0·99	2·875	6
6	$HOOCCH_2$—[d]	0·96 ± 0·09	0·97	3·359	9
7	H_3CCH_2—	0·54 ± 0·07	0·97	2·775	5
8	$HOOCCH_2CH_2$—	0·77 ± 0·05	0·99	3·450	5

[a] No *2* is ethyl mercaptoacetate; [b] correlation coefficient; [c] number of derivatives; [d] data for the calculation of k taken from ref.[20], see text to Table III.

TABLE V

Rate Constants of the Reactions of Phenyl Isothiocyanate with Different Nucleophilic Agents and the Dissociation Constants of these Substances

Nucleophil	Reactive form	pK_a^a 25°C	k_{25} $M^{-1}s^{-1}$	k_i/k_0
Sodium hydroxide	HO^-	13·99[b]	0·119[c]	1·0
Methanol	RO^-	15·09[d]	0·480[c]	4·0
Ethanol		15·93[d]	0·650[e]	5·5
Phenylalanine	RNH_2	9·18[f]	0·146[g]	1·2
Lysine		9·18	0·133	1·1
Serine		9·21	0·083	0·7
Glutamic acid		9·47	0·110	0·9
Valine		9·72	0·155	1·5
Leucine		9·74	0·160	1·3
Glycine		9·78	0·243	2·0
Alanine		9·87	0·129	1·1
Sodium sulphide	HS^-	7·10	13·2	111·0
Ethyl mercaptoacetate	RS^-	7·93	128·0	1 182·0
1,4-Dithiothreitol		8·25[h]	275·0	2 310·0
Methyl 3-mercaptopropionate		9·33	2 245·0	18 890·0
2-Mercaptoethanol		9·48	607·0	5 100·0
Mercaptoacetic acid		10·11	1 900·0[i]	15 980·0
Ethanethiol		10·35	492·0	4 140·0
3-Mercaptopropionic acid		10·40	2 770·0	23 250·0
Benzenethiol		7·35	1 320·0	11 090·0

[a] For thiols determined spectrophotometrically, for the sulphide average value of spectrophotometric and kinetic measurements; [b] from ref.[38]; [c] from ref.[39]; [d] from ref.[40]; [e] from ref.[41]; [f] for all amino acids from ref.[42]; [g] for all amino acids from ref.[33]; [h] from ref.[12]; [i] from ref.[20].

Spectrophotometric determination of dissociation constants pK_a. The pK_a values of sodium sulphide (dissociation constants of first degree) and thiols were determined by spectrophotometric titration in the McIlvain 0·2M citrate–phosphate and Sörensen 0·2M borate buffer solutions at 25°C according to the method of Benesh[11], using the wavelength region 230—265 nm, the concentration of the sulphide or thiols being 30—500 μM. In the case of ethanethiol, ethylmercaptoacetate, and methyl 2-mercaptopropionate the aqueous buffer solutions contained 1 vol.% of methanol. The results of measurements are compiled in Table V.

Kinetic determination of dissociation constants pK_a. The kinetic determination of K_a value was done by measuring the rate constant k_{obs} (s^{-1}) for the reactions of C_6H_5NCS with the sulphide. The measurements were performed with reaction mixtures buffered at pH values ranging from pH 6·5 to pH 11·0 at 25°C at the wavelength 295 nm. The kinetic measurements and calculation of k_{obs} were performed exactly according to the procedure described in the chapter Kinetic measurements. The initial concentrations of C_6H_5NCS, thiol, and the sulphide were 31·3, 50

and 920 μM, respectively. The first dissociation constant of H_2S was calculated from the modified form of the Henderson–Hasselbach equation (Eq. (*4*)):

$$k_{obs} = [K_a(k_b - k_{obs})/c_{H^+}] + k_a \,, \qquad (4)$$

where k_a and k_b (s^{-1}) are the rate constants for the reaction of the undissociated or dissociated form of H_2S with C_6H_5NCS, respectively. A more detailed derivation of this method of pK_a determination can be found in literature[19].

RESULTS AND DISCUSSION

Isothiocyanates react with the dissociated thiol groups of the R—SH compound studied and Na_2S, *i.e.* with their RS^- or HS^- form. This statement is in agreement with the generally accepted idea of extremely different nucleophility of dissociated

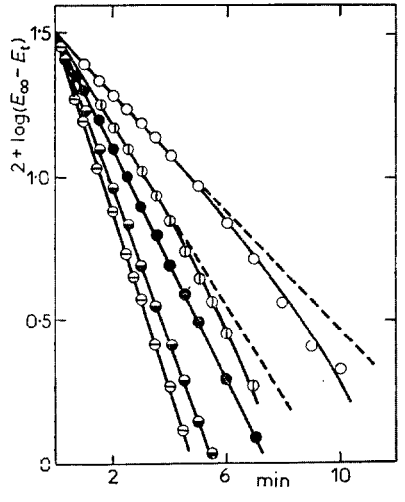

FIG. 1

Kinetics of the Reactions of Phenyl Isothiocyanate with Sodium Sulphide in Buffered Reaction Mixtures as a Function of pH

Curves: ○ pH 6·5 (16·70), ◑ pH 7·0 (13·20), ● pH 7·5 (12·40), ◒ pH 8·0 (13·25), ⊖ pH 9·0 (13·0), ⊖ pH 10·0 (12·85), ⊖ pH 11·0 (12·82). The calculated values of k ($M^{-1}\,s^{-1}$) are written in parentheses. Initial concentration C_6H_5NCS 31·3 μM, and sodium sulphide 920 μM, respectively. Ionic strength 0·1, 25°C. Full lines express the data found experimentally, dashed lines express the expected theoretical course.

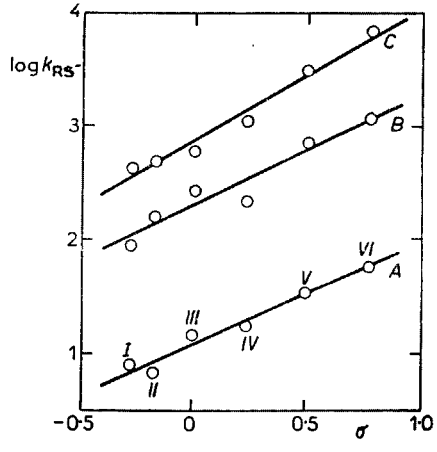

FIG. 2

Relationship between log k for the Reactions of the 4-Substituted Phenyl Isothiocyanates with Sodium Sulphide *A*, Dithiothreitol *B*, and 2-Mercaptoethanol *C* and the Hammett Constants σ

Designation of the derivatives as in Table II, σ according to ref.[30].

and undissociated thiol groups. One of the evidence for the statement consists in the fact that the pH-dependence of the rate constant k_{obs} for the reaction of ITC with these compounds shows the shape of dissociation curve. The first such revelation ensued from the study of the reactivity of ITC with respect to mercaptoacetate[20]. In the course of this study analogous reactions concerning other investigated thiols were found. As illustration, we shall present at least some results concerning the reaction of C_6H_5NCS with sodium sulphide (Table I). The values of the rate constants k_{HS^-} ($M^{-1}\,s^{-1}$) determined in buffered reaction mixture the pH values of which varied in the pH range from 7·0 to 11·0 are practically equal. The presented values of k_{obs} (s^{-1}) are a linear function of the variable $(k_b - k_a)/c_{H^+}$. The slope of this relationship is equal to $K_a = 5{\cdot}60 \,.\, 10^{-8} M^{-1}$ and represents the first dissociation constant of H_2S ($pK_a = 7{\cdot}25 \pm 0{\cdot}03$). This relationship is also characterized by the calculated value of correlation coefficient $r = 0{\cdot}98$ and a good agreement of the calculated value of pK_a with that one found experimentally (see Experimental). In view of a relatively low reactivity of HS^- ion with respect to C_6H_5NCS in comparison with RS^- compounds there was a favorable opportunity of measuring the rate constant in a relatively wide pH range. However, Fig. 1 shows that the kinetic investigation of the reaction with HS^- ions is impaired by the effect of the decomposition of the product, *i.e.* N-phenyl dithiocarbamate. This decomposition occurring with various N-monosubstituted dithiocarbamates (DTC) was studied in more detail earlier[21–24]. DTC differ from the esters of DTC (EDTC) which are products of other investigated reactions by their instability in acid medium. But the decomposition yielding ITC (refs[25–29]) is characteristic of both the types of these reactions. Owing to a convenient and constant analytical concentration of dissociated forms (first order reaction) ITC reacted quantitatively and the products were stable under the conditions used for the investigation of the reactions of ITC with HS^- and RS^- ions. That means that in the case of DTC (Eq. (*A*)) as well as EDTC (Eq. (*B*)) the decomposition rate with corresponding rate constant k_{-1} was negligible. The reactions were investigated at $pH < pK_a$ of thiols and addition products (Tables II and III).

$$HS^- + R^1{-}NCS \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} R^1{-}NH{-}C(=S)(-\overset{\ominus}{S}) \qquad (A)$$

$$R^2S^- + R^1{-}NCS \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} R^1{-}N(-\overset{\ominus}{S}){-}C(=S){-}S{-}R^2 \qquad (B)$$

$$+H^+ \uparrow\downarrow -H^+ \qquad -H^+ \uparrow\downarrow +H^+$$

$$R^2SH \qquad R^1{-}NH{-}C(=S){-}S{-}R^2$$

The dissociation of EDTC (Eq. (*B*)) was proved and characterized for different types of compounds (combined R^1 and R^2) which made possible also to determine the values of k_{-1} (ref.[29]).

The reactions of benzyl isothiocyanate and 4-substituted derivatives of C_6H_5NCS with HS^- ions and different RS^- compounds are universally of the type Ad_N which is confirmed by the fact that the Hammett equation is obeyed (Table IV). The slopes with a positive ϱ varying in a narrow range of values. Only ethanethiol shows a lower value of ϱ. In the relation, Fig. 2 allows to illustrate the differences between the reactivities of C_6H_5NCS with respect to HS^- ions and certain RS^- compounds. At the same time, it indicated the possibility of correlating the values of log k for the reaction of ITC with one of the nucleophilic agents to the corresponding values obtained with arbitrary other agents. This also holds for other types nucleophilic agents, *e.g.* OH^- ions and amines in the same manner as it was shown in the papers where the reactivities of ITC with respect to OH^- ions, glycine, and mercaptoacetate[31] have

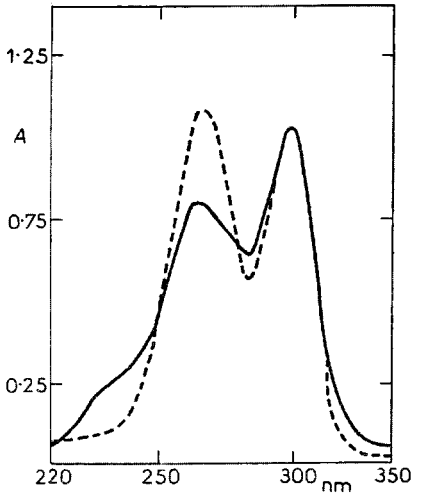

FIG. 3
UV Absorption Spectra of the Products of the Reactions of Isothiocyanates (31·3 μM) with Ethyl Mercaptoacetate (125 μM)
Curves: ——— phenyl, — — — benzyl isothiocyanate. Cell width 10 mm.

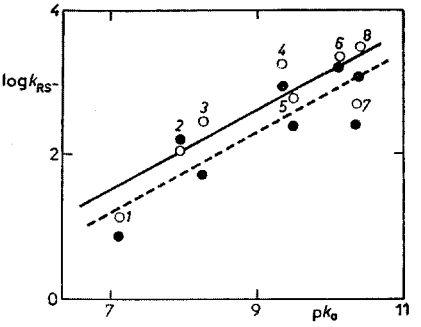

FIG. 4
log k for the Reactions of Phenyl and Benzyl Isothiocyanates with Sodium Sulphide and Aliphatic Thiols as a Function pK_a of These Thiol Compounds
Curves: ○ full line C_6H_5NCS; $\varrho = 0{\cdot}54 \pm \pm 0{\cdot}05$ ($r = 0{\cdot}88$), dashed line $C_6H_5CH_2$. .NCS; $\varrho = 0{\cdot}56 \pm 0{\cdot}05$ ($r = 0{\cdot}86$). *1* sodium sulphide, *2* ethyl mercaptoacetate; designation of the other thiols as in Table III.

been mutually correlated[20,32,33]. The reaction with mercaptoacetate is still more interesting because this species is added to alkyl- and aryl ITC which is succeeded by a consecutive cyclization to rhodanines. According to the pH of reaction mixture (in alkaline region) rhodanines may decompose and yield either EDTC or ITC and thiol[34–37]. The investigation of the reaction of C_6H_5NCS with ethyl mercaptoacetate has shown that addition is also succeeded by a cyclization reaction which yields quantitatively the corresponding rhodanine as the only reaction product (Fig. 3).

Fig. 4 shows the relationship between the log of the rate constants of the reactions of C_6H_5NCS and $C_6H_5CH_2NCS$ with different thiols and the corresponding pK_a values. In spite of values of the correlation coefficients of these relationships (0·88 and 0·86, respectively) which may be due to the heterogeneity of thiols, these relationships demonstrate the importance of pK_a values for the estimation of the rate constants of thiols.

The values of the rate constants of the reactions C_6H_5NCS with various hitherto studied nucleophilic substances are presented in Table V. These data enable us to compare their nucleophilities and point out that their different nucleophility cannot be explained by the differences in the basicity of OH^- ions, amines, and thiols. The high reactivity of ITC with respect to RS^- compounds which has been revealed is another evidence for the correctness of the idea that the reactions of natural and synthetic ITC with protein SH groups will be also of the greatest importance for the elucidation of the mechanisms of biological effects of these substances. Furthermore, the rate constant of the reaction of $C_6H_5CH_2NCS$ with benzenethiol is also included in Table V. In the course of the investigation of the reaction of $C_6H_5CH_2NCS$ and derivatives of C_6H_5NCS with other substituted benzenethiols there was also a need to change the reaction conditions and the methods of kinetic investigations which emphasizes the differences in the character of thiol groups bonded to aliphatic and aromatic radical.

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