SPECIALIA

Reactions of cysteine, its derivatives, glutathione, coenzyme A, and dihydrolipoic acid with isothiocyanates

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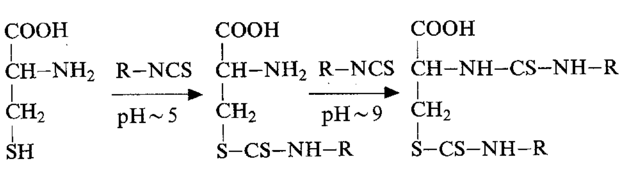
Summary. Isothiocyanates react with deprotonated SH groups of investigated compounds to give the esters of N-monosubstituted dithiocarbamic acid. In the presence of the SH and NH_2 groups (Cys, GSH), isothiocyanates react primarily with the SH groups. The reactions are dependent on the $pK_{a\,SH}$ values.

Isothiocyanates are used in protein chemistry as reagents for the determination of primary structure of peptides and proteins. These substances with antimicrobial and cytotoxic effect are also known as outstanding 'SH reagents'[1-7]. In this study we investigated the reactivity of isothiocyanates with cysteine, its derivatives, glutathione (GSH), CoASH, and dihydrolipoic acid as biologically important substances.

Material and methods. N-Acetyl-L-cysteine, 6,6′-dithiodinikotinic acid (CPDS) and DTNB were provided by Calbiochem. The other SH compounds were purchased by Lachema, Brno. Isothiocyanates were prepared according to Antoš et al.[8] and Dyson et al.[9]. The concentration of SH compounds were determined with DTNB.

The kinetic measurements were carried out under conditions of pseudomonomolecular reaction in 0,2 M citrate-phosphate buffer. The concentrations of SH compounds were $5.65 \cdot 10^{-7}$–$3.96 \cdot 10^{-5}$ M (R–S$^-$), isothiocyanates $3.13 \cdot 10^{-5}$–$1 \cdot 10^{-4}$ M. The rate constants were carried out spectrophotometrically and with CPDS by using of the following pK_a values: Cys 8.29; N-acetyl cysteine 9.52; methyl ester of cysteine 6.50; GSH 8.63; cysteamine 8.35; dihydrolipoic acid 10.70; CoASH 9.60[10,11].

Results and discussion. Isothiocyanates react with the deprotonated SH groups of thiols to give the esters of N-monosubstituted dithiocarbamic acids. The rate constants of the 1st order reaction (min^{-1}) are dependent on pH and change according to the form of the dissociation curve (table 1). The constants obtained of the 2nd order reaction k ($M^{-1} \cdot sec^{-1}$) vary in the pH region 4.5-5.5 merely in the range of admissible deviations, but at higher pH values, the NH_2-groups of Cys, GSH and cysteamine start to manifest themselves in the reactions. Cys represents a complicated zwitterion system[12,13]. In buffered solutions at pH 4-6, isothiocyanates react primarily with the SH group of Cys. After change of pH to the alkaline region, present isothiocyanates react also with free NH_2 group. The additional products of SH-group are stable in the presence of Cys. However, they decompose at equimolar concentrations to give isothiocyanates which react with the free NH_2-group in consecutive reaction.



$$\begin{array}{lcl} \text{COOH} & & \text{COOH} & & \text{COOH} \\ | & & | & & | \\ \text{CH–NH}_2 & \text{R–NCS} & \text{CH–NH}_2 & \text{R–NCS} & \text{CH–NH–CS–NH–R} \\ | & \xrightarrow[\text{pH}\sim 5]{} & | & \xrightarrow[\text{pH}\sim 9]{} & | \\ \text{CH}_2 & & \text{CH}_2 & & \text{CH}_2 \\ | & & | & & | \\ \text{SH} & & \text{S–CS–NH–R} & & \text{S–CS–NH–R} \end{array}$$

On the basis of this evidence, it may be explained that Todrick and Walker[14], investigating the reaction between allyl isothiocyanate and Cys in alkaline medium, obtained only 1 product, i.e. N-substituted derivative. By the conversion of S-[N(fenyl) thiocarbamoyl] cysteine (concentration $1 \cdot 10^{-4}$ M, t = 30 °C, 0.2 M phosphate buffer) into its

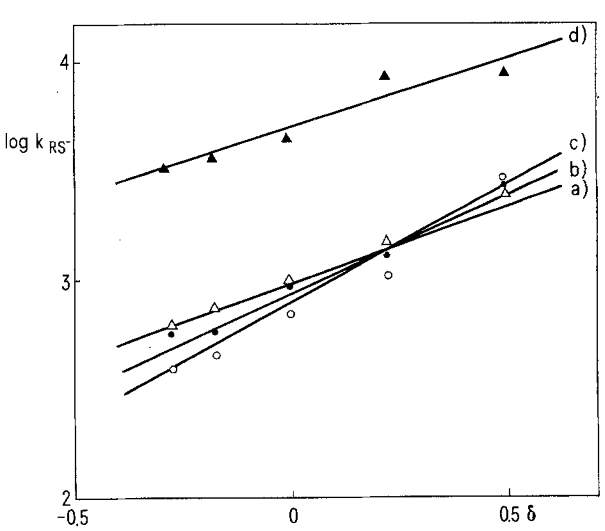


Fig. 1. Relationship between logk and the Hammett constants ρ for the reactions of 4-substituted phenyl isothiocyanates with Cys (A), GSH (B), cysteamine (C) and dihydrolipoic acid (D).

Table 1. Rate constants of the 1st and 2nd order reactions of benzyl isothiocyanate and 4-substituted phenyl isothiocyanate with cysteine as a function of pH

No.	R–NCS	k(min^{-1})			k(M^{-1} · sec^{-1})		
	pH	4.5	5.0	5.5	4.5	5.0	5.5
I.	Benzyl	0.008	0.036	0.115	2.05	2.92	2.96
II.	4-Methoxyphenyl	0.016	0.059	0.235	4.10	4.63	6.05
III.	p-Tolyl	0.024	0.076	0.300	6.15	6.17	7.77
IV.	Phenyl	0.025	0.118	0.405	6.43	9.59	10.43
V.	4-Bromphenyl	0.042	0.120	0.462	10.80	9.75	11.90
VI.	4-Acetylphenyl	0.092	0.331	0.986	23.68	26.91	25.39
VII.	4-Nitrophenyl	0.267	0.606	1.136	68.67	49.26	29.26

Table 2. Rate constants k(M^{-1} · sec^{-1}) of the reactions of isothiocyanates with cysteine, its derivatives, GSH, dihydrolipoic acid, and CoASH (Denotation of the derivatives is given in table 1)

Compound	Concentration (M)				k(M^{-1} · s^{-1})					
	pK$_a$	pH	S$^-$	R–NCS	I	II	III	IV	V	VI
N-acetyl cysteine	9.52	7.6	$1.5 \cdot 10^{-5}$	$3.13 \cdot 10^{-5}$	5.35	7.23	10.73	13.26	15.25	22.63
Cysteine methyl ester	6.50	4.5	$3.96 \cdot 10^{-5}$	$1.00 \cdot 10^{-4}$	0.39	0.60	0.74	0.86	1.35	4.04
Cysteamine	8.35	4.5	$5.65 \cdot 10^{-7}$	$1.00 \cdot 10^{-4}$	4.01	4.13	4.72	9.73	12.68	27.44
GSH	8.63	6.5	$1.00 \cdot 10^{-5}$	$3.13 \cdot 10^{-5}$	2.52	5.97	6.06	7.32	10.68	28.91
Dihydrolipoic acid*	10.70	6.5	$5.04 \cdot 10^{-7}$	$1.00 \cdot 10^{-4}$	32.07	33.39	37.36	44.97	83.66	87.96
CoASH	9.60	6.5	$3.60 \cdot 10^{-6}$	$1.00 \cdot 10^{-4}$	2.78	–	–	4.20	–	–

* pK$_a$ value is referred to both SH groups.

N-substituted analogue, we obtained the following rate constants:

pH	k(min^{-1})	t/2 (min)
5.70	0.062	11.15
6.28	0.260	2.65
6.72	0.598	1.16

As in the case of other SH compounds, the investigated reactions are nucleophilic additions, because the reactivity with respect to the SH group increases with decreasing density of Π-electrons on the carbon atom of the NCS-group, which is due to change of the carbon rest of the substituents. This is evidenced by the validity of the Hammett equation (figure 1).

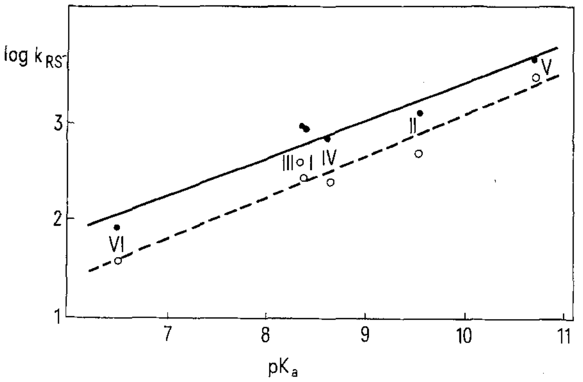


Fig. 2. Reaction of phenyl- and benzyl isothiocyanates with Cys (I), N-acetyl cysteine (II), cysteamine (III), GSH (IV), dihydrolipoic acid (V) and cysteine methylester (VI). log k as a function of the pK$_a$ values of these SH compounds (full line, phenylisothiocyanate; $\rho = 0.43$, $r = 0.97$; dashed line, benzyl isothiocyanate; $\rho = 0.38$, $r = 0.96$).

The values of the rate constants for the reactions of the derivatives of Cys, GSH, and dihydrolipoic acid with benzyl isothiocyanate and substituted phenylisothiocyanates are given in table 2. The reactivity of the SH-groups of these substances is proportional to the pK$_a$ values (figure 2). The rate constants obtained for CoASH do not obey that relationship, which might be explained by the more complicated structure of its molecule in comparison with other SH-compounds.

The results obtained enable us to have an idea of the differences in the reactivities of the individual thiols, and at the same time may be good for the study of the interactions between the SH-groups of proteins and isothiocyanates.

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