KINETICS OF REACTIONS OF ISOTHIOCYANATES WITH DIGLYCINE, CYSTINE, LYSINE, α-N-ACETYLLYSINE AND OXIDISED GLUTATHIONE

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The kinetics of nucleophilic addition reactions of substituted phenyl isothiocyanates and benzyl isothiocyanate with amino groups of diglycine, cystine, oxidised glutathione, α-N-acetyllysine and lysine was studied. The rate constants, measured under conditions of pseudomonomolecular reactions, make it possible to compare the reactivity of the amino groups with their pK_a values. In the case of lysine the rate constants for both its amino groups were determined using phenyl [^{35}S]-isothiocyanate.

Our previous papers[1-6] dealt with the kinetics of reactions of aromatic isothiocyanates with amino acids, ethanethiol, mercapto acids, N-acetylcysteine and glutathione. The aim of the present study is to investigate the reactivity of isothiocyanates with amino groups of further amino acids and peptides. We studied therefore the reactivity of selected isothiocyanates with diglycine, α-N-acetyllysine, lysine and oxidised glutathione (GSSG). The first two compounds contain one free amino group, whereas cystine and GSSG contain two amino groups which are equivalent concerning the reaction with isothiocyanates. On the other hand, lysine has two nonequivalent amino groups with different pK_a values. Lysine reacts with phenyl isothiocyanate under formation of N,N-α,ε-bis(phenylthiocarbamoyl)lysine (*C*). This reaction can proceed *via* two intermediates, N-α-phenylthiocarbamoyllysine (*A*) and/or N-ε-phenylthiocarbamoyllysine (*B*). The first intermediate (*A*) was isolated and subjected to a further reaction with phenyl isothiocyanate.

EXPERIMENTAL

Chemicals and instruments. Phenyl [^{35}S] isothiocyanate, activity 20 Ci/M (Amersham), 1-phenyl-4-[(ε-phenylthiocarbamoyl)aminobutyl]-2-thiohydantoin (*E*) (Fluka), α-N-acetyllysine (Calbiochem), lysine and GSSG (Lachema) were commercial products. Other isothiocyanates, 1-phenyl-4-(ε-aminobutyl)-2-thiohydantoin (*D*), and the compound *A* were prepared according to the literature[7-9,10].

The reaction rates were measured spectrophotometrically on a Perkin-Elmer 402 instrument at 25°C. The pH values of the buffer solutions were adjusted electronically on a Radiometer 4 pH-meter. The pK_a values for α-N-acetyllysine and the intermediate *A* were determined potentiometrically at 25°C in an aqueous medium in the presence of 2% of methanol (Autotitrator Radiometer). The activity of the products of reaction of phenyl ^{35}[S] isothiocyanate with lysine was measured on a Tesla NRB-212 radiochromatograph and on an NZQ-714 instrument. Linear correlations were calculated on an Olivetti 101 computer using the least squares method.

Methods of measurement. The kinetic measurements were performed under conditions for a pseudomonomolecular reaction in a 0·1M borate buffer containing 2% of dioxane; concentration of the amino acid or peptide was 2—10 mM, concentration of isothiocyanate 0·045—0·150 mM. The overall rate of the reaction of phenyl [^{35}S] isothiocyanate with both amino groups of lysine was followed spectrophotometrically. After the reaction had ended, the intermediate *A* and the product *C* were transformed into the respective thiohydantoins *D* and *E* by treatment with HCl. The reaction mixture was subjected to radiochromatographic analysis on Silufol (Kavalier) thin-layer plates, using *p*-xylene–methanol (1 : 1) system. The R_F values of the compounds *E* and *D* were determined (UV light, $\lambda = 254$), and compounds were further analysed radiochromatographically as [^{35}S] labelled products. The activity was measured on a planchet after cutting out the spots. The apparent rate constants, k_{obs} (min^{-1}), were calculated from the spectrophotometrically obtained constants k_{obs}, using the relationship $k_{obs} = k_{obs\,\alpha-NH_2} + k_{obs\,\varepsilon-NH_2}$; the ratio k_{obs} for the α- and ε-amino groups corresponds to the ratio of activities of the 2-thiohydantoins *D* and *E* because the intermediate *B* was analytically not found.

The actual rate constants k[mol^{-1} s^{-1}] were calculated using the following pK_a values: diglycine 8·17, cystine 8·80, GSSG 9·65, lysine 8·95 (α-NH_2), 10·53 (ε-NH_2) (ref.[11-13]). For α-N-acetyllysine we obtained $pK_a = 10·50$. These values were found at 25°C for an aqueous medium (1—2% of methanol). We assume that both the amino groups in cystine and GSSG are equivalent. Under the same conditions, the pK_a value for the ε-amino group in the isolated intermediate *A* was found to be 10·61.

RESULTS AND DISCUSSION

The results of the kinetic measurements of the reaction of phenyl isothiocyanates and benzyl isothiocyanate with diglycine, cystine and GSSG are given in Table I. Isothiocyanates react with amino acids and peptides under formation of N-thioureido carboxylic acids. This reaction is used *e.g.* in degradation of peptides by the Edman method[14]. These reactions are nucleophilic additions of the type Ad_N in which the carbon atom of the NCS group is attacked. The reaction rate depends on the character of the substituent. Whereas in the case of isothiocyanates the reaction is accelerated by electron-withdrawing substituents (a greater electron deficit at the carbon of the —NCS group), in the case of amino acids the reaction is slowed down (lower basicity of the amino nitrogen). This is proved by a positive value of the slope ϱ in the Hammett equation, observed in the reaction of diglycine with substituted phenyl isothiocyanates ($\varrho = 0·71 \pm 0·06$, correlation coefficient $r = 0·985$). These facts are in accord with our previous investigations concerning the kinetics of reactions of aryl and aralkyl isothiocyanates with amino acids[1-3].

Comparison of rate constants for the reactions of amino acids and peptides with isothiocyanates shows that the reaction rates of these addition reactions depend not only on the reactivity of isothiocyanates but also on the character of the corresponding nucleophilic compounds. We tried to correlate the rate constants k [$mol^{-1}\ s^{-1}$] with the pK_a values of the studied compounds including also glycine and butylamine (the rate constants for the latter two compounds were already determined previously[1,15]). We found a linear correlation between $\log k$ and pK_a values of the studied compounds ($\log k = 0{\cdot}297\ pK_a - 3{\cdot}560$; $r = 0{\cdot}964$). This correlation makes it possible to estimate the reactivity of amino groups in various amino compounds in their reactions with phenyl isothiocyanate on the basis of their pK_a values. Since

TABLE I

Values of k [$mol^{-1}\ s^{-1}$] and $t/2$ [min] for the Reactions of R-NCS with Derivatives of Amino Acids in 0·1M Borate Buffer (pH 9·8) at 25°C

Derivative R	Starting molar concentration		$t/2$	$k \cdot 10^2$
	derivative	R-NCS		
		Diglycine		
Benzyl	$5 \cdot 10^{-3}$	$5 \cdot 10^{-5}$	51·7	4·5 ± 0·4
4-Methoxyphenyl	$5 \cdot 10^{-3}$	$5 \cdot 10^{-5}$	42·9	5·5 ± 0·4
4-Methylphenyl	$5 \cdot 10^{-3}$	$5 \cdot 10^{-5}$	41·6	5·7 ± 0·5
Phenyl	$5 \cdot 10^{-3}$	$5 \cdot 10^{-5}$	33·7	7·1 ± 0·4
4-Bromophenyl	$5 \cdot 10^{-3}$	$5 \cdot 10^{-5}$	22·1	10·7 ± 0·6
4-Acetylphenyl	$5 \cdot 10^{-3}$	$5 \cdot 10^{-5}$	14·2	16·6 ± 0·7
		Cystine		
Benzyl	$1 \cdot 10^{-3}$	$4{\cdot}5 \cdot 10^{-5}$	231·0	3·2 ± 0·2
Phenyl	$1 \cdot 10^{-3}$	$5{\cdot}0 \cdot 10^{-5}$	46·2	13·2 ± 0·4
4-Bromophenyl	$1 \cdot 10^{-3}$	$5{\cdot}0 \cdot 10^{-5}$	34·7	18·7 ± 0·9
		GSSG		
Benzyl	$5 \cdot 10^{-3}$	$1{\cdot}5 \cdot 10^{-4}$	77·0	5·3 ± 0·7
Phenyl	$1 \cdot 10^{-3}$	$5{\cdot}0 \cdot 10^{-5}$	138·6	15·1 ± 0·7
4-Bromophenyl	$1 \cdot 10^{-3}$	$5{\cdot}0 \cdot 10^{-5}$	99·0	19·6 ± 0·9
		α-N-Acetyllysine		
Phenyl	$2{\cdot}7 \cdot 10^{-3}$	$7{\cdot}4 \cdot 10^{-6}$	75·3	36·5 ± 0·6

TABLE II

Rate Constants for the Reactions of Phenyl [^{35}S]Isothiocyanate (Concentration $7{\cdot}38 \cdot 10^{-6}$M) with Lysine (Concentration $2{\cdot}68 \cdot 10^{-3}$M) in 0·1M Borate Buffer

pH	M[a]	k_{obs} min^{-1}	$t/2$ min	$k \cdot 10^2$ $mol^{-1}\,s^{-1}$
		α-Amino group		
8·33	$5{\cdot}18 \cdot 10^{-4}$	0·0039	177·7	12·8
8·92	$12{\cdot}94 \cdot 10^{-4}$	0·0098	70·7	12·6
9·80	$23{\cdot}47 \cdot 10^{-4}$	0·0181	38·3	12·8
		ε-Amino group		
8·33	$0{\cdot}18 \cdot 10^{-4}$	0·0004	1 733·0	39·7
8·92	$0{\cdot}64 \cdot 10^{-4}$	0·0016	433·2	42·0
9·80	$4{\cdot}20 \cdot 10^{-4}$	0·0105	66·0	41·6
9·80[b]	$3{\cdot}59 \cdot 10^{-4}$[b]	0·0095[b]	72·9[b]	44·1[b]

[a] Molar concentration of the reacting form; [b] the same concentration of N-α-phemylthocarbamoyllysine was used instead of lysine.

under the conditions of the measurements the carboxyl group is ionised the decrease in basicity of the amino groups in peptides is caused mainly by the electron-withdrawing effect of the carbonyl groups in the peptide bonds. Therefore, the basicity of an amino group in a peptide increases with its increasing distance from the carbonyl group as seen in the case of diglycine and GSSG (pK_a = 8·17 and 9·65, respectively). We can thus understand that also the reactivity of the amino group in cystine and ot the α-amino group in lysine will be closer to the reactivity of GSSG rather than to that of diglycine.

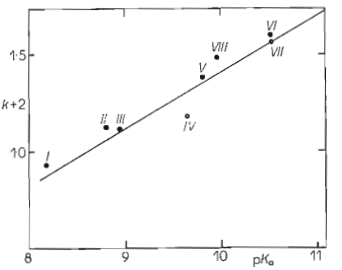
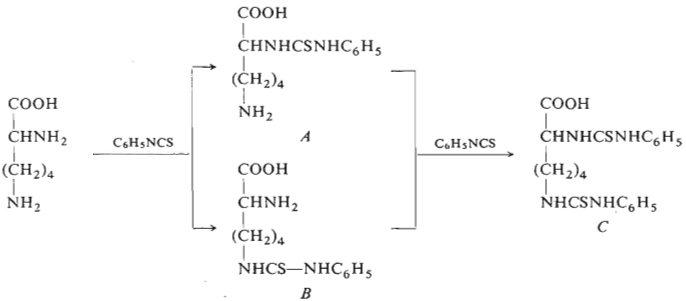


FIG. 1

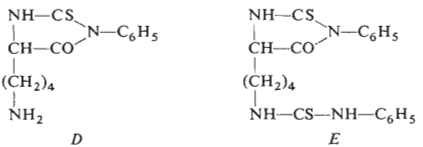
Plot of log k [$mol^{-1}\,s^{-1}$] for the Reaction of Amino Acids and Peptides with Phenyl Isothiocyanate (pH 9·80) against Their pK_a Values

I Diglycine, *II* cystine, *III* α-amino group of lysine, *IV* GSSG, *V* glycine, *VI* ε-amino group of lysine, *VII* α-N-acetyllysine, *VIII* n-butylamine ($\varrho = 0{\cdot}297$, $r = 0{\cdot}964$).

The results of the kinetic measurements of the reactions of phenyl [^{35}S]isothiocyanate with lysine and with the intermediate *A* are given in Table II. The reaction can be described by Scheme 1. The molar concentrations of the reacting forms



SCHEME 1



and the reactivity of both amino groups of lysine depend on their pK_a values which are in this case different. The reactivity of a particular amino group will therefore depend on the pH of the reaction medium. The fact that our analytical studies did not prove any presence of the intermediate *B*, and also the measured reaction half-lives, lead us to the conclusion that at pH 8·33–9·80 the reaction is specific only for the α-amino group of lysine. This conclusion is confirmed also by the identical rate constants for the reaction of phenyl isothiocyanate with the ε-NH_2 group of lysine, the intermediate *A* and α-N-acetyllysine, as well as by the pK_a value found for the intermediate *A* (pK_a = 10·61) which corresponds to the pK_a value of the ε-amino group of lysine (pK_a = 10·53). Under the given conditions lysine and the intermediate *A* will react concurrently with phenyl isothiocyanate.

The obtained results can be used in the study of interactions isothiocyanate–protein, particularly for the estimation of the reactivity of free amino groups.

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