BIOCHEMICALLY IMPORTANT REACTIONS OF 2-FURYLETHYLENES. CHARACTERIZATION OF THE REACTIVITY TOWARDS THIOLS

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Reactions of 1-(5-R^1-2-furyl)-2-R^2,R^3-ethylenes with thiols were investigated in aqueous solutions at pseudomonomolecular conditions and 25°C. The obtained kinetic dependences made it possible to calculate the second order rate constants (1 mol^{-1} s^{-1}). The reactivity of the studied 2-furyl-ethylenes increases with the increasing electron-accepting effect of substituents varying both in position 5 of the furan and 2 of the exocyclic double bond. The reactivity of thiols towards 2-furyl ethylenes was found to raise with their increasing basicity.

As shown in our preceding paper[1], 2-furylethylenes exhibit a remarkable ability to react with low-molecular thiols; the reacting site of the thiolate anion was, at least with 2-furylethylenes, the more electrophilic $C_{(1)}$ atom of the exocyclic double bond in these nucleophilic addition reactions. The reaction of 2-furylethylenes proceeds also with SH groups of high-molecular thiol, especially with thiolproteins both *in vitro* (experiments with purified model thiol enzymes[2,3]) and *in vivo* (inactivation of the key SH enzymes of glycolysis[2,4]). Modification of SH groups of thiolproteins, having catalytical functions in the cell, particularly SH enzymes involved in the energy yielding processes (glycolysis, oxidative phosphorylation) is of decisive importance from the standpoint of the mechanism of antimicrobial and cytotoxic effects of 2--furylethylenes[2,4,5]. Since "biological response" is, in general, a function of affinity of a bioactive substance to receptoric sites in the cell[6] (frequently expressed by the reactivity towards the corresponding modified functional groups), we characterized the reactivity of 2-furylethylenes towards low-molecular thiols.

EXPERIMENTAL

Chemicals. The purity of benzylmercaptan, cysteine, dithiothreitol, glutathione, coenzyme A, 2-mercaptoethanol, 2-aminoethanethiol hydrochloride, and 2-mercaptoacetic acid was determined prior to use with Ellman reagent[7].

UV and VIS spectra of 2-furylethylenes and products of their reactions with thiols were measured with a Specord UV VIS (Zeiss, Jena) spectrophotometer in a 0·2 mol l^{-1} buffer solutions according to Clark and Lubs (pH 4—6, ref.[8]) with 0·5% dioxane in the 220—500 nm region.

Kinetic measurements were monitored with a UV and VIS spectrophotometer SP 30 (Pye-Unicam), with a temperated cell bed at 25°C. The buffer solutions were adjusted with the pH-meter OP 207 (Radelkis, Hungary). The stock solutions of 2-furylethylenes were prepared with dioxane, those of thiols in water or in methanol. Reaction mixtures contained 0·5% (v/v) of dioxane, in some cases the same percentage of methanol. Buffer solutions (0·2 mol l^{-1}) according to Clark and Lubs were employed for preparation of reaction mixtures; the pH of these was verified before and after the reaction. The initial concentrations of 2-furylethylenes and thiols were 5 . 10^{-5} mol . . l^{-1} and 1,2 and 3 . 10^{-3} mol l^{-1}, respectively. Under these conditions (at least a 20-fold excess of the thiol with respect to 2-furylethylene) the reactions proceeded as pseudomonomolecular. In the UV and VIS regions of spectra, in which the reaction kinetics were examined, only 2-furylethylene and products of its reaction with thiol absorbed; therefore, it was possible to determine the first order rate constants k_{obs}(s^{-1}) according to[9] from the line slope of linear dependence log $|A_t - A_{t+\Delta t}|$ on time using equation (1):

$$\log |A_t - A_{t+\Delta t}| = \log |(A_0 - A_\infty)(1 - e^{-k_{obs}\Delta t})| - k_{obs}(\ln 10)^{-1} t. \qquad (1)$$

A in this equation stands for absorbance and the subscript for time, Δt is an properly chosen time interval. The second order rate constants k(l mol^{-1} s^{-1}) were calculated according to equation (2):

$$k = k_{obs}(K_a + c_{H^+}) c_0^{-1} K_a^{-1}, \qquad (2)$$

where K_a is the dissociation constant of the thiol, c_0(mol l^{-1}) its initial analytical concentration and c_{H^+}(mol l^{-1}) the concentration of H$^+$ ions. The rate constants are the mean value of three measurements at various concentrations of the thiol. Their standard deviation from the mean value varies within 5—10%.

RESULTS AND DISCUSSION

2-Furylethylenes exert a characteristic maximum in buffered aqueous solutions in the 310—380 nm region. Thiols under investigation do not absorb in this region. Due to mutual reaction, noticeable spectral changes of the reaction mixture take place, as seen in Fig. 1 examplifying the reaction of 1-(5-nitro-2-furyl)-2-nitroethylene (*IX*) with 2-mercaptoethanol. Similarly as with this example, also other derivatives under study (Table I) form reaction products having a lowered and towards lower wavelengths shifted absorption maximum. These spectral changes are in agreement with the proposed reaction mechanism of 2-furylethylenes with thiols as reported in our previous paper[1] (Scheme A):

I—X (see Table I)

TABLE I

The first (k_{obs}) and second order (k) rate constants determined for the reaction of 1-(5-R^1-2-furyl)--2-R^2,R^3-ethylenes with 2-mercaptoacetic acid at 25°C. The reactions were investigated in the Clark–Lubs buffer of pH 5·0

Compound	R^1	R^2	R^3	k_{obs}^a s^{-1}	k_{obs}^b s^{-1}	k_{obs}^c s^{-1}	k $l\ mol^{-1}\ s^{-1}$
I	H	NO_2	$COOCH_3$	$2·3 . 10^{-2}$	$4·7 . 10^{-2}$	$7·0 . 10^{-2}$	$3·9 . 10^6$
II	CH_3	NO_2	$COOCH_3$	$2·0 . 10^{-2}$	$4·0 . 10^{-2}$	$6·0 . 10^{-2}$	$3·3 . 10^6$
III	I	NO_2	$COOCH_3$	$5·8 . 10^{-2}$	$1·7 . 10^{-1}$	$1·7 . 10^{-1}$	$9·6 . 10^6$
IV	Br	NO_2	$COOCH_3$	$4·9 . 10^{-2}$	$9·9 . 10^{-2}$	$1·5 . 10^{-1}$	$8·2 . 10^6$
V	$COOCH_3$	NO_2	$COOCH_3$	$9·0 . 10^{-2}$	$1·8 . 10^{-1}$	$2·7 . 10^{-1}$	$1·5 . 10^7$
VI	NO_2	NO_2	$COOCH_3$	$2·4 . 10^{-1}$	$>3 . 10^{-1}$	$>3 . 10^{-1}$	$4·0 . 10^7$
VII	NO_2	$COOC_2H_5$	$COOC_2H_5$	$1·1 . 10^{-3}$	$2·2 . 10^{-3}$	$3·3 . 10^{-3}$	$1·8 . 10^5$
VIII	NO_2	COOH	H	$6·0 . 10^{-5}$	$1·2 . 10^{-4}$	$1·8 . 10^{-4}$	$1·0 . 10^4$
IX	NO_2	NO_2	H	$3·5 . 10^{-2}$	$7·0 . 10^{-2}$	$1·0 . 10^{-1}$	$5·8 . 10^6$
X	H	NO_2	H	$2·5 . 10^{-3}$	$5·1 . 10^{-3}$	$7·6 . 10^{-3}$	$4·2 . 10^5$

^{a}Concentration of the thiol 1, ^{b} 2, ^{c} 3 . 10^{-3} mol l^{-1}.
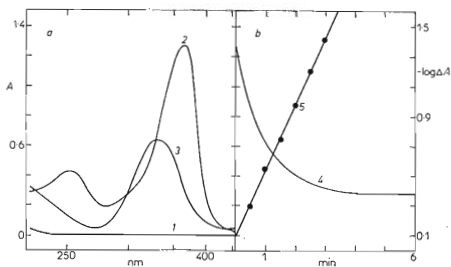

FIG. 1

Electron absorption specta (A) of 2-mercaptoethanol (1), 1-(5-nitro-2-furyl)-2-nitroethylene (2), their reaction mixture after reaction (3), and the kinetics of the reaction (B) monitored at 363 nm — dependence A_t (4) and log ΔA (5) upon time. The initial concentration of 2-furylethylene 5 . 10^{-5} mol l^{-1}, that of thiol 1 . 10^{-3} mol l^{-1}. The spectra and kinetics were measured in an acetate buffer of pH 5·0 at 25°C; cell width 1 cm. $\Delta A = A_t - A_{t+\Delta t}$, $\Delta t = 4$ min

Spectrometry has been, therefore, a suitable tool for examination the reaction of 2-furylethylenes with thiols and consequently, it was utilized for the kinetic study.

These reactions have in buffered solutions of pH 4—6 and a 20fold excess of the thiol in regard to 2-furylethylene a quantitative course and their kinetics can be described by the equation valid for the first order reactions. The pseudomonomolecular course of these reactions evidences not only the linear dependence of log ΔA values on time (Fig. 1), but also the direct proportionality between the determined first order rate constants and concentrations of the thiol used (Table I).

Thiols undergo reaction with 2-furylethylenes under the given conditions in a dissociated form. This statement is in line with the generally accepted concept[10,11] concerning the extremely different nucleophility of the dissociated and undissociated forms of SH groups. One proof of this statement follows from the dependence of the first order rate constant $k_{obs}(s^{-1})$ upon the pH of the buffered reaction medium at a constant analytical concentration of the thiol. Thus, this dependence for the reaction of ethylene *IX* with 2-mercaptoethanol is seen in Fig. 2. The dependence follows the shape of a dissociation curve, unfortunately incomplete due to a high reactivity at higher pH values unmeasurable by this means. Taking the RS^- form of the thiol into account, the real (pH independent) second order rate constants $k(1 \text{ mol}^{-1} \text{ s}^{-1})$ were obtained from the observed first order rate constants (Table II).
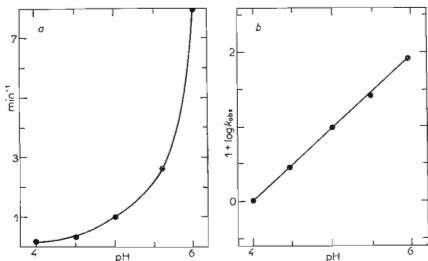

FIG. 2

Dependence of the first order rate constant $\dot{k}_{obs}$ on pH for the reaction of 1-(5-nitro-2-furyl)-2-
-nitroethylene (*IX*) with 2-mercaptoethanol. Clark–Lubs buffers[8], initial concentrations of 2-furylethylene $5 . 10^{-5}$ mol l^{-1}, of thiol $1 . 10^{-3}$ mol l^{-1}, temperature 25°C

Reactivity of thiols is a function of their basicity; this follows from an experiment in which the second order rate constants for reactions of ethylene *IX* with thiols of very different pK_a constants were determined. These constants together with dissociation constants of thiols are summarized in Table III. The relationship between these two quantities is plotted in Fig. 3, the mathematic relation is expressed by equation (3):

$$\log k = 0.783 \, pK_a - 1.512 . \tag{3}$$

TABLE II

Rate constants of the reaction of 1-(5-nitro-2-furyl)-2-nitroethylene (*IX*) with 2-mercaptoethanol in the Clark–Lubs buffers of various pH values. The initial concentrations of 2-furylethylene $5 . 10^{-5}$ mol l^{-1}, of 2-mercaptoethanol $1 . 10^{-3}$ mol l^{-1}, temperature 25°C

pH	k_{obs}, s^{-1}	c_{RS^-}, mol l^{-1}	k, l mol^{-1} s^{-1}
4·0	$1·6 . 10^{-3}$	$1·9 . 10^{-9}$	$8·4 . 10^5$
4·5	$4·5 . 10^{-3}$	$6·0 . 10^{-9}$	$7·5 . 10^5$
5·0	$1·6 . 10^{-2}$	$1·9 . 10^{-8}$	$8·4 . 10^5$
5·5	$4·4 . 10^{-2}$	$6·0 . 10^{-8}$	$7·3 . 10^5$
6·0	$1·3 . 10^{-1}$	$1·9 . 10^{-7}$	$6·8 . 10^5$

TABLE III

Dissociation constants of thiols (pK_a) and second order rate constants (k) determined for reactions of the corresponding thiols with 1-(5-nitro-2-furyl)-2-nitroethylene (*IX*) at 25°C. Reactions were investigated in the Clark–Lubs buffer of pH 4·4

Thiol	pK_a (ref.)	k, l mol^{-1} s^{-1}
Cysteine	8·15 (12)	$7·08 . 10^4$
2-Aminoethanethiol	8·35 (13)	$1·95 . 10^5$
Glutathione	8·56 (12)	$1·26 . 10^5$
Dithiothreitol	9·12 (14)	$3·02 . 10^5$
Benzylmercaptan	9·43 (8)	$6·58 . 10^5$
Coenzyme A	9·60 (13)	$9·30 . 10^5$
2-Mercaptoethanol	9·72 (15)	$7·77 . 10^5$
2-Mercaptoacetic acid	10·22 (12)	$5·80 . 10^6$

The correlation coefficient of equation (3) $r = 0.955$ $(F = 62.820, n = 8)$. The lower correlation coefficient value can be due to a considerable heterogeneity of thiols and/or various sources of pK_a constants. In spite of these facts the above-mentioned dependence demonstrates the importance of pK_a for the reactivity prediction of various thiols to 2-furylethylenes.

Finally, Table I summarizes rate constants determined for reactions of all 2-furyl-ethylene derivatives under study with 2-mercaptoacetic acid as a model thiol. The reactions were investigated in a Clark–Lubs buffer of pH 5·0, at initial concentration of 2-furylethylenes $5 . 10^{-5}$ mol l^{-1}. The first order rate constants k_{obs} were determined for three various concentration of thiol (1, 2 and $3 . 10^{-3}$ mol l^{-1}). Comparison of their values with the corresponding concentrations of the thiol made it possible to ascertain that reactions of all 2-furylethylenes with 2-mercaptoacetoacetic acid proceeded as pseudomonomolecular under the given conditions and consequently, the determined values k_{obs} are suitable for calculation of the second order rate constants. These real rate constants $k(1 \text{ mol}^{-1} \text{ s}^{-1})$ enabled us to make some con-
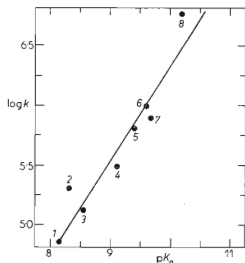

FIG. 3

Dependence of logarithms of second order rate constants $k(1 \text{ mol}^{-1} \text{ s}^{-1})$ determined for reactions of 1-(5-nitro-2-furyl)-2-nitro-ethylene (IX) with various thiols upon pK_a values of their SH group at 25°C. *1* Cysteine, *2* aminoethanethiol, *3* reduced glutathione, *4* dithiothreitol, *5* benzylmercaptan, *6* co-enzyme A, *7* 2-mercaptoethanol, *8* 2-mer-captoacetic acid
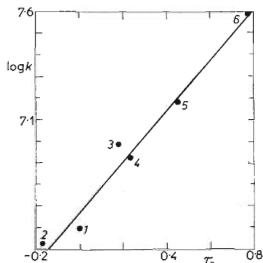
FIG. 4

Dependence of logarithms of second order rate constants determined for reactions of 1-(5-R¹-2-furyl)-2-nitro-2-methoxycarbonyl-ethylenes *I—VI* (Table I) with 2-mercapto-acetic acid upon Hammett σ_p constants of substituents R^1 at 25°C

clusions concerning the relation between the structure of 2-furylethylenes and their reactivity towards thiols. 1-(5-R^1-2-Furyl)-2-nitro-2-methoxycarbonylethylenes $I - VI$ reveal an increase in reactivity towards thiols with the increasing electron-accepting effect of the substituent R^1 in position 5 of the furan ring. This influence can be quantitatively expressed by the Hammett constants σ_p, since the electron with drawal occurs in this case through the partly aromatic furan ring. The dependence of reactivity of ethylenes $I - VI$ upon Hammett σ_p constants (taken from ref.[16]) of substituents R^1 (Fig. 4) is expressed by equation (4):

$$\log k = 1 \cdot 218 \sigma_p + 6 \cdot 646 \quad (r = 0 \cdot 972; \text{ F} = 68 \cdot 427; n = 6).\tag{4}$$

The fact that the line slope of this dependence has a positive value, together with the finding that thiols are added in dissociated form to $C_{(1)}$ atom of the exocyclic double bond of furylethylenes provided evidence that these reactions are of the Ad_N type. This statement is in accord with the fact that the reactivity of other derivatives under study $(VII - X)$ to thiols raise with the increasing electron-accepting effect of substituents at the exocyclic double bond. Reaction products of derivatives II, III, V and VI (Table I) with thiols have so far not been identified. Basing on the observed Hammett dependence one is entitled to presume that, similarly as with derivatives $I, IV, VII - X$, the nucleophillic addition to $C_{(1)}$ of the exocyclic double bond was involved.

It is worth noting that virtually all derivatives under investigation possess a comparable, some of them even a much higher reactivity toward thiols than the classical and in biochemistry mostly employed SH reagent N-ethylmaleinimide and the Ellman reagent. The rate constants of these substances, determined under analogous conditions as with 2-furylethylenes for reactions with cysteine were found to be $3 \cdot 1 \cdot 10^4$, and $4 \cdot 1 \cdot 10^4 \text{ l mol}^{-1} \text{ s}^{-1}$, respectively, (ref.[11]).

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