KINETIC ANALYSIS OF REACTIONS OF PHENYLHYDRAZONOPROPANEDINITRILES WITH THIOLS

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Phenylhydrazonopropanedinitriles react with aliphatic thiols in aqueous solutions to give 2-phenylhydrazono-3-imino-3-alkylthiopropanedinitriles. The observed first-order rate constants k_{obs} (s^{-1}) depend on pH value of the reaction medium and on the thiol concentration. Kinetic analysis showed that the reactions are nucleophilic additions of bimolecular type involving non-dissociated phenylhydrazonopropanedinitriles and thiolate anions as the electrophilic and nucleophilic partners, respectively. A relation is given enabling transformation of the found k_{obs} constants into the second-order rate constants k ($l\ mol^{-1}\ s^{-1}$) independent on the pH value of medium and of the thiol concentration.

Phenylhydrazonopropanedinitriles (carbonyl cyanide phenylhydrazones[1]) are known as highly efficient uncouplers of oxidative and photosynthetic phosphorylation[2-6]. Although these compounds serve as a tool in investigation of the above-mentioned and other bioenergetic processes[7-13], molecular mechanism of their action is not well understood yet. Most frequently mentioned are two ideas about the mechanism of the uncoupling effect of phenylhydrazonopropanedinitriles. One of them considers these substances to act as *trans*-membrane carriers of protons and ions[14], the other supposes that they modify the membrane proteins[15-17]. Hence it is presumed that efficiency of phenylhydrazonopropanedinitriles is determined by their lipophilic and acido–basic properties and chemical reactivity. The present communication describes a way of characterization of reactivity of phenylhydrazonopropanedinitriles by means of the second-order rate constants of their reactions with thiols. Thiols were chosen for the reaction partners, because SH groups of the membrane proteins play an important role in the mechanism of oxidative and photosynthetic phospho-

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rylation[18-22] and are considered to be receptors of phenylhydrazonopropanedinitriles[7,17,23]. A characterization of reactivity, acid–base and lipophilic properties of a suitably chosen series of phenylhydrazonopropanedinitriles could make it possible to confront these properties with the uncoupling effect and to elucidate its nature.

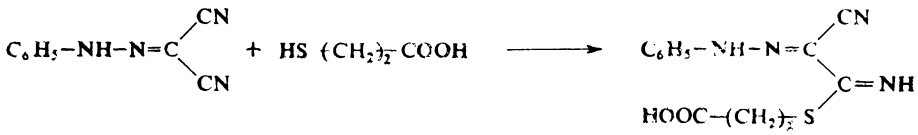
EXPERIMENTAL

The kinetics of reactions of phenylhydrazonopropanedinitriles with the model thiols were followed spectrophotometrically by measuring time changes of the absorbance corresponding to the greatest difference in the absorption spectra of the starting and final compounds (Fig. 1). The initial concentrations of reactants were below the respective solubilities in the given medium. Stock solutions of phenylhydrazonopropanedinitriles and thiols were prepared in methanol and water, respectively. The reaction solutions contained at most 1% methanol and were buffered with the Clark–Lubs phthalate (pH 4—6), phosphate (pH 6—8), and borate (pH 8—10) buffers[24]. The reactions were followed continuously after mixing the two reactants, the thiol to phenylhydrazonopropanedinitrile ratio being at least 20 : 1. At these conditions the reactions obeyed the first-order kinetics (Fig. 2). The first order rate constants k_{obs} (s^{-1}) were read as the slope of the time dependence of ln $(A_\infty - A_t)$, where A_∞ and A_t represent the absorbances of the reaction solution after completed reaction and at a time t, respectively. Each constant was obtained as a mean value of three kinetic measurements with experimental error of 1—5%. The second-order rate constants k ($l\ mol^{-1}\ s^{-1}$) were calculated from the relation derived in the present communication and using values of the dissociation constants of the thiols and phenylhydrazonopropanedinitriles[17,25].

Phenylhydrazonopropanedinitrile was obtained by diazotization of aniline and coupling with malononitrile[1]. The spectrophotometric measurements were carried out with an SP 30 UV-VIS (Pye Unicam, Cambridge, England) and a Specord UV-VIS (Zeiss, Jena, GDR) spectrophotometers.

RESULTS AND DISCUSSION

Phenylhydrazonopropanedinitriles react with thiols in aqueous medium to give addition products, the new C— S bond being formed at one or at both cyano groups[26]. So *e.g.* in case of the reaction of phenylhydrazonopropanedinitrile with 3-mercaptopropanoic acid the product is 3-(1-imino-2-cyano-2-phenylhydrazonoethylthio)-propanoic acid:



The addition products are decomposed into the original components in aqueous medium in the absence of sufficient excess of one of the reactants[17]. The excess

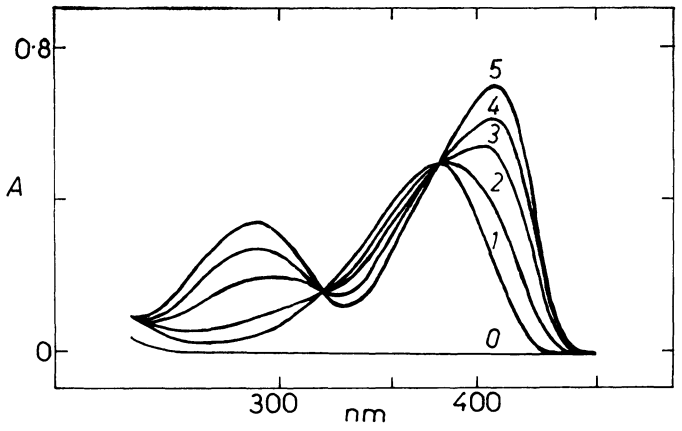


FIG. 1

Spectrophotometric indication of the reaction of phenylhydrazonopropanedinitrile with 3-mercaptopropanoic acid in phosphate buffer of pH 7·0 at 25°C. The initial concentrations: phenylhydrazonopropanedinitrile $2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}, 3-mercaptopropanoic acid $1 \cdot 10^{-3}$ mol l^{-1}. Curve *0* represents the spectrum of the thiol, *1* spectrum of phenylhydrazonopropanedinitrile. Curves *2*, *3*, *4*, *5* represent spectra of the reaction mixture after 60, 150, 240, 600, 1 200 s, respectively, from the reaction start

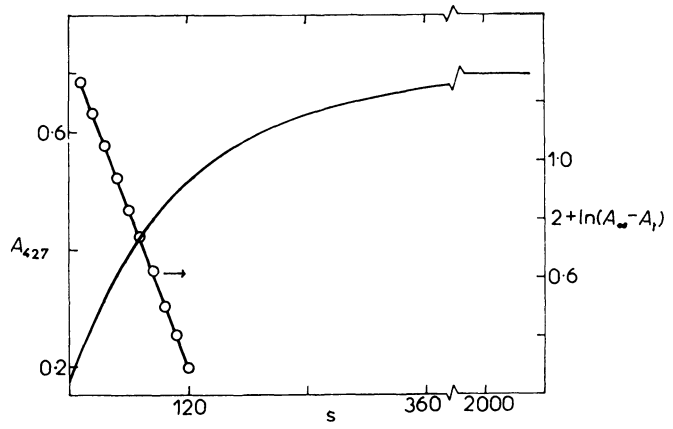


FIG. 2

Kinetics of the reaction of phenylhydrazonopropanedinitrile with 3-mercaptopropanoic acid in phosphate buffer of pH 7·0 at 25°C. Initial concentrations: phenylhydrazonopropanedinitrile $2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}, 3-mercaptopropanoic acid $1{\cdot}0 \cdot 10^{-3}$ mol l^{-1}. The thiol solution served as the blank

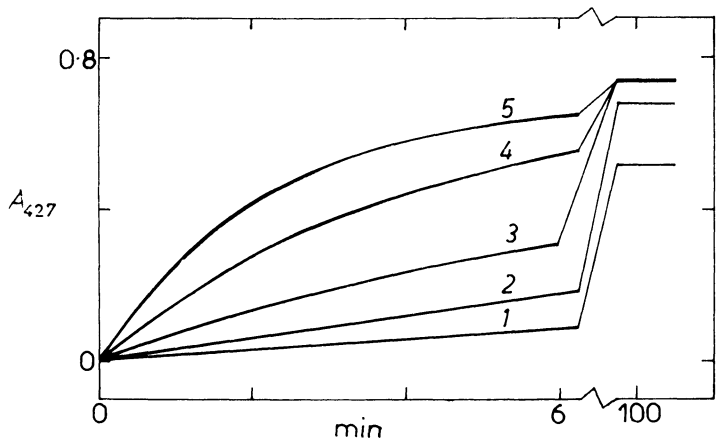


FIG. 3

The reaction kinetics of phenylhydrazonopropanedinitrile with 3-mercaptopropanoic acid at various concentrations of the thiol in phosphate buffer of pH 6·05 at 25°C. Initial concentrations: phenylhydrazonopropanedinitrile $2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}, 3-mercaptopropanoic acid $1 \cdot 10^{-4}$ *1*, $2 \cdot 10^{-4}$ *2*, $4 \cdot 10^{-4}$ *3*, $1 \cdot 10^{-3}$ *4*, and $2 \cdot 10^{-3}$ *5* mol l^{-1}. The solution of phenylhydrazonopropanedinitrile ($2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}) was used as the blank

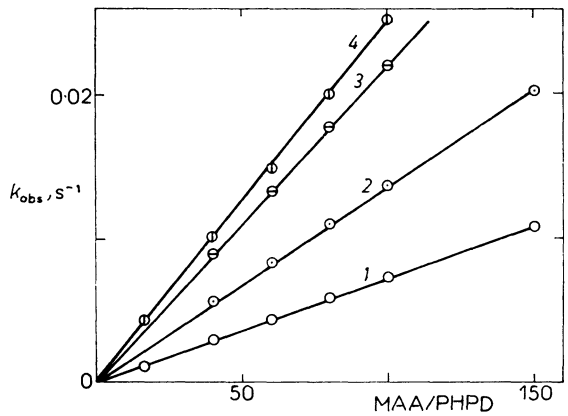


FIG. 4

Dependence of the 1. order rate constants k_{obs} (s^{-1}) of the reaction of phenylhydrazonopropanedinitrile (PHPD) with 3-mercaptopropanoic acid (MPA) on the pH value of medium and on the thiol excess (MPA/PHPD). The reaction medium: phosphate buffers of pH 6·05 *1*, 6·50 *2*, 7·00 *3*, 8·10 *4*. Temperature 25°C. Initial concentration of phenylhydrazonopropanedinitrile was $2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}

of thiol thus affects not only the reaction rate but also the conversion degree of phenylhydrazonopropanedinitriles into the addition products. Fig. 3 represents the reaction kinetics of phenylhydrazonopropanedinitrile with 2-mercaptoacetic acid followed at various concentrations of this thiol. A more than 99% conversion into the addition product takes place with a more than 20 fold molar excess of the thiol. The calculated first-order rate constant k_{obs} depends on the thiol excess and on the pH value of the medium (Fig. 4). The pH dependence of log k_{obs} has a concave shape, which indicates the fact that the reaction involves the non-dissociated phenylhydrazonopropanedinitriles and thiolate anions. The reaction is fastest at such pH value which ensures the optimum concentration of the reactive forms of the two reaction partners. In media of lower pH values the concentration of the reactive (non-dissociated) form of phenylhydrazonopropanedinitrile increases (up to the value practically equal to the analytical concentration of the reactant), but, at the same time, that of the reactive (ionized) form of the thiol decreases. At higher pH values the situation is reversed (Fig. 5). From these findings it can be concluded that reactivities of various phenylhydrazonopropanedinitrile derivatives with thiols can only be compared in media ensuring equal concentrations of their non-dissociated forms. One possibility is to follow the reaction in media of various pH values equal to the respective dissociation constants (pH = pK_a) of the individual derivatives[17]. Another possibility consists in following the reaction at a single pH value far below the dissociation constants (pH $\ll$ pK_a) of the respective derivatives: in this case the phenylhydrazonopropanedinitriles are only present in their non-ionized forms. The second-order rate constants (independent of pH and concentrations of the reactants)

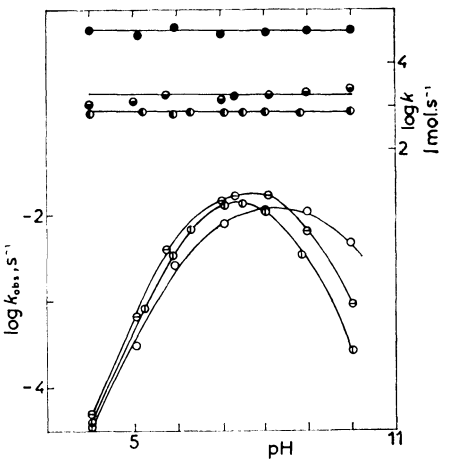


FIG. 5

Dependence of logarithm of the 1. order rate constants (log k_{obs}, empty circles) and of logarithm of the 2. order rate constants (log k, full circles) on the pH values of the reaction medium for the reaction of phenylhydrazonopropanedinitrile with 3-mercaptopropanoic acid (○, ●), cysteine (⊖ ◒), and 2-mercaptoethylamine (⊙ ◐). Initial concentrations: phenylhydrazonopropanedinitrile $2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}, thiols $1 \cdot 10^{-3}$ mol . . l^{-1}. Temperature 25°C

can then be calculated from the simple relation $k = k_{obs}/c_{RS^-}$. Further aim of this work, however, was to find a general relation enabling characterization of reactivity of phenylhydrazonopropanedinitriles to thiols by the 2. order reaction rate constants on the basis of kinetic data obtained by measurements in media of any arbitrary pH value. To simplify the evaluation of the results, the measurements were carried out with the following presumptions: *1*) Conversion of one of the reactants into the addition product must be greater than 99%, and the reaction course must obey the 1. order kinetics. *2*) The rate constants of the dissociation reactions of both the phenylhydrazonopropanedinitriles and thiols must be greater than that of the reaction itself by several orders of magnitude. The dissociation reactions thus do not limit the rate of the reaction proper, but only determine concentrations of the reactive forms of reactants. *3*) The reaction must take place at a constant proton concentration at which the reaction rate depends on concentration of the non-dissociated form of phenylhydrazonopropanedinitriles and dissociated form of thiols. The first condition is fulfilled at high excess of one of the reactants. The second condition is fulfilled in our case, because the dissociation rate constants, in general, are substantially higher than $10^2 - 10^3\ s^{-1}$, the observed rate constants of the reactions of phenylhydrazonopropanedinitriles with thiols varying within the limits from 10^{-5} to $10^{-2}\ s^{-1}$. The third condition is fulfilled by carrying out the reactions in buffer solutions.

At the above-mentioned conditions the reactions of phenylhydrazonopropanedinitriles with thiols are described by the following scheme in which AH = phenylhydrazonopropanedinitrile, RS^- = thiolate anion, and $HASR^-$ = ionized form of the addition product:

$$\begin{array}{ccccc} AH & + & RS^- & \xrightarrow{k} & HASR^- \\ k_{dA} \uparrow\downarrow k'_{dA}K_A & & k_{dRS} \uparrow\downarrow k'_{dRS}K_{RS} & & \\ A^- + H^+ & & RSH - H^+ . & & \end{array}$$

Hence, the reaction rate can be expressed by the relation:

$$v = k \,.\, c_{AH} \,.\, c_{RS^-} \,. \qquad (1)$$

As c_{RS^-} = const. at the conditions of $c_{RSH} \gg c_{AH}$, c_{H^+} = const., and $k_d \gg k_{obs}$, the relation is simplified to

$$v = k' \,.\, c_{AH} \,. \qquad (2)$$

The concentrations of the reactive forms of the two reactants (c_{AH}, c_{RS^-}) can be expressed by means of their dissociation constants (K_A, K_{RS}) and analytical con-

centrations (c_A, c_{RS}):

$$K_A = c_{A^-} \cdot c_{H^+}/c_{AH} \tag{3}$$

$$K_{RS} = c_{RS^-} \cdot c_{H^+}/c_{RSH} \tag{4}$$

$$v = k' c_{H^+} \cdot c_A/(K_A + c_{H^+}) \tag{5}$$

$$k_{obs} = k' \cdot c_{H^+}/(K_A + c_{H^+}) \tag{6}$$

$$v = k_{obs} \cdot c_A \,. \tag{7}$$

Linearization of the integral form of time dependence of concentrations of the reactants gave the values of the rate constant k_{obs}. This 1. order rate constant k_{obs}. can be transformed into the 2. order rate constant independent of pH of the medium for which the k_{obs} was calculated from the kinetic data

$$k = k_{obs}(K_A + c_{H^+})(K_{RS} + c_{H^+})/(c_{RS} \cdot c_{H^+} \cdot K_{RS}) \,. \tag{8}$$

Hence, with Eq. (8) it is possible to convert the k_{obs} determined at any arbitrary pH values of medium into the 2. order rate constants independent of pH. Fig. 5

TABLE I

Values of the 1. order (k_{obs}) and the 2. order rate constants (k) determined at various pH values for the reactions of phenylhydrazonopropanedinitrile (PHPD) and its 3-Cl (CPHPD) and 4-OCF_3 derivatives (FPHPD) with 3-mercaptopropanoic acid (MPA). Initial concentrations: PHPD $1 \cdot 10^{-5}$ mol l^{-1}, CPHPD and FPHPD $2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}, thiol $1{\cdot}0 \cdot 10^{-3}$ mol l^{-1}. Temperature 25 C. The pK_a constants of PHPD (ref.[17]) derivatives 6·55, 6·00, and 6·00, pK_a of MPA (ref.[25]) 10·22

pH	PHPD + MPA		FPHPD + MPA		CPHPD + MPA	
	k_{obs}, s^{-1}	k, l mol^{-1} s^{-1}	k_{obs}, s^{-1}	k, l mol^{-1} s^{-1}	k_{obs}, s^{-1}	k, l mol^{-1} s^{-1}
4·0	$4{\cdot}0 \cdot 10^{-5}$	$6{\cdot}4 \cdot 10^{4}$	$7{\cdot}5 \cdot 10^{-5}$	$1{\cdot}2 \cdot 10^{5}$	$7{\cdot}6 \cdot 10^{-5}$	$1{\cdot}2 \cdot 10^{5}$
5·0	$2{\cdot}7 \cdot 10^{-4}$	$4{\cdot}4 \cdot 10^{4}$	$4{\cdot}5 \cdot 10^{-4}$	$8{\cdot}1 \cdot 10^{4}$	$1{\cdot}0 \cdot 10^{-3}$	$1{\cdot}8 \cdot 10^{5}$
6·0	$3{\cdot}0 \cdot 10^{-3}$	$6{\cdot}4 \cdot 10^{4}$	$3{\cdot}7 \cdot 10^{-3}$	$1{\cdot}2 \cdot 10^{5}$	$4{\cdot}9 \cdot 10^{-3}$	$1{\cdot}6 \cdot 10^{5}$
7·0	$7{\cdot}3 \cdot 10^{-3}$	$4{\cdot}6 \cdot 10^{4}$	$5{\cdot}6 \cdot 10^{-3}$	$1{\cdot}0 \cdot 10^{5}$	$8{\cdot}2 \cdot 10^{-3}$	$1{\cdot}5 \cdot 10^{5}$
8·0	$1{\cdot}0 \cdot 10^{-2}$	$5{\cdot}0 \cdot 10^{4}$	$4{\cdot}8 \cdot 10^{-3}$	$8{\cdot}2 \cdot 10^{4}$	—	—
9·0	$1{\cdot}1 \cdot 10^{-2}$	$5{\cdot}4 \cdot 10^{4}$	—	—	—	—
10·0	$6{\cdot}4 \cdot 10^{-3}$	$4{\cdot}8 \cdot 10^{4}$	—	—	—	—

gives the pH dependences of the 2. order rate constant calculated from Eq. (*8*) and from the k_{obs} rate constants determined for the reaction of phenylhydrazonopropanedinitrile with 3-mercaptopropanoic acid, 2-mercaptoethylamine, and cysteine. From the Fig. 5 it is evident that the k values are pH independent. This fact also follows from Table I summarizing the k_{obs} values of the reaction of the parent phenylhydrazonopropanedinitrile and its 3-Cl and 4-OCF_3 derivatives with 3-mercaptopropionic acid along with the k values calculated from k_{obs} according Eq. (*8*).

For the reactions of phenylhydrazonopropanedinitriles with high excess of thiols, *i.e.* in cases when the solutions contain practically only the non-dissociated form of the phenylhydrazonopropanedinitrile (pH $\ll$ pK_a of the respective derivative), complex Eq. (*8*) is simplified to Eq. (*9*):

$$k = k_{obs} \cdot c_{H^+}(c_{H^+} + K_{RS})/(c_{H^+} \cdot c_{RS} \cdot K_{RS}) = k_{obs}/c_{RS^-} \cdot \qquad (9)$$

Another simplification of Eq. (*8*) for pH < pK_a of the both reaction partners gives the known dependence between log k_{obs} and pH values of the reaction medium, Eq. (*11*).

$$k = k_{obs} \cdot c_{H^+}/(c_{RS} \cdot K_{RS}), \quad c_{H^+} \gg K_A, \quad c_{H^+} \gg K_{RS} \qquad (10)$$

$$\log k_{obs} = \text{const.} + \text{pH} \qquad (11)$$

From Fig. 5 it really follows that in acidic media (pH < 6) the dependence of log k_{obs} *vs* pH is practically linear with the slope close to 1 which follows from Eq. (*11*).

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