ANTIPHAGE EFFECT OF ETHYLENIMINES

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The use of the bacteriophage model for the study of radiomimetic compounds has led to an assumption of a presence of correlation between the antiphage and antimitotic effects of these substances. It was established that monofunctional compounds, e.g., monoethylenimine-derived agents exert an antimitotic effect when they are introduced in doses which exceed 50-100 times those of polyethylinimines [5, 6, 7]. However it was shown that the activity of certain monoethylenimines may be equated with their bifunctional analogs. In particular this is true of ethoxen, whose antitumor activity was unexpectedly found to be higher than that of some bis- and polyethylenimines [3].

The above data provided a basis for a comparative study of the antiphage effects of mono-, di-, tri-, and tetraethylenimines.

EXPERIMENTAL METHODS

The following compounds were studied: ethylenimine, 1-ethylenimino-2-oxybuten-3(ethoxen), N,N-malonyl-bis-ethylenimine (MEI-2)*, (1) N',N",N"'-tri(ethylene)-triamide-thiophosphoric acid (thiophosphamide), tetra-(ethylenamide)-1,4-piperazinediphosphoric acid (dipin). The antiphage effect of the above compounds was studied in relationship to the T group phages (T1, T2, T6 and T7) of E. coli. Test tubes containing 0.9 ml of solvent with a corresponding dose of each compound received 10^7 phage particles in a volume of 0.1 ml. After a given exposure a series of dilutions were made in which the number of phage particles which retained their infectivity was determined by the method of agar layering.

EXPERIMENTAL RESULTS

Solutions of all the compounds under study, prepared in acid buffers, had pronounced antiphage properties. The degree of their inhibitory effect depended on the pH value of the solution, time of action of this compound on the phage, and in some cases on the phage itself (Tables 1, 2 and 3).

As seen in Table 1 the effect of all the compounds studied was more pronounced at acid pH values. The activity of ethylenimine, thiophosphamide and dipin was almost completely lost at pH 7.2, while ethoxen and MEI-2 retained their inhibiting properties. The maximal antiphage effect with thiophosphamide and dipin was noted at 37°C, while the temperature of the medium had no significant effect on the activity of ethoxen and of MEI-2.

Ethoxen and MEI-2 had an antiphage effect at concentrations lower than those of other ethylenimines (Table 2). Ethylenimine, ethoxen and dipin exerted a selective action on odd-numbered coliphages. Even-numbered phages were relatively stable against these compounds. MEI-2 and thiophosphamide did not have this selective effects and had an inhibiting effect on all the bacteriophages studied to approximately the same degree (Table 3).

Thus, the above mentioned ethylenimine-derived compounds had dissimilar antiphage effects, which is in accordance with their differential antitumor activities. Ethoxen and MEI-2 were most active at normal pH values.

* Differences in the chemical structure of MEI and MEI-2 have not yet been determined although they differ in their antiphage activities. The biological activity and synthesis of MEI have been described by us earlier.

TABLE 1. The Relationship of the Antiphage Effect of Ethylenimines to the pH of the Buffer Solution (phage T1, exposure 30 min, temperature 37°C)

Preparation	Concentration in moles	Distilled water	Acitate buffer		Phosphate buffer	
			pH			
			5,0	5,6	6,1	7,2
Ethylenimine	$1\cdot10^{-2}$	2	0,04	1	8	88
	$1\cdot10^{-3}$	50	55	80	85	100
Ethoxen	$1\cdot10^{-2}$	60	0	0	0	0,06
	$1\cdot10^{-3}$	82	0,001	0,001	0,002	8
MEI-2	$1\cdot10^{-2}$	51	0	0	0,001	1
	$1\cdot10^{-3}$	80	0,02	0,02	2	65
Thiophosphamide	$1\cdot10^{-2}$	70	0	1,8	13	90
	$1\cdot10^{-3}$	100	2,1	25	50	100
Dipine	$1\cdot10^{-2}$	78	0	0,43	6,5	85
	$1\cdot10^{-3}$	100	1,5	18	100	100

Note: In this and the following tables the figures represent the number of infective phage particles (per cent of control) which remained after treatment with compounds under study.

TABLE 2. The Relationship of the Antiphage Activity of Ethylenimines to Their Concentration (phage T1, acetate buffer at pH 5.0, exposure 30 min)

Preparation	Concentration of ethylenimines in moles						
	1×10^{-2}	5×10^{-3}	$2{,}5\times10^{-3}$	$1{,}2\times10^{-3}$	6×10^{-4}	3×10^{-4}	1×10^{-4}
Ethylenimine	0,04	2	31	80	100		
Ethoxen	0	0	0	0,001	2,3	33	41
MEI-2	0	0	0	0,003	3,8	40	74
Thisphosphamide	0	0,0005	0,07	1	7,8	25	100
Dipine	0	0,0008	0,05	1,2	8	43	100

Ethoxen is of special interest in the determination of correlation between its antimitotic and antiphage effects. As noted previously, this preparation had a high antimitotic activity but at the same time it proved to be a strong inhibitor of extracellular phage. In order to explain the mechanism of action of radiomimetic substances a hypothesis of cross bonds [4] had been proposed. According to this hypothesis one molecule of nitrous yprite, ethylenimine and of other compounds which have an alkylizing effect, reacts with two electron-donor centers of a biological macromolecule, such as DNA. This property may be possessed only by bi- and polyfunctional compounds. In this case how can the high biological activity of ethoxen, which has one alkylizing group connected to an ethylenimine ring be explained? If the above hypothesis is to be followed it must be supposed that in the case of ethoxen the second reaction apparently is achieved through the unsaturated ethylene and hydroxyl groups which are contained within the molecule.

MEI-2 also is of interest from the point of view of the possibility of utilizing the bacteriophage model in order to select new antitumor agents. This new compound with its still unknown antitumor activity had a strong antiphage effect. A comparison of the antiphage activity of MEI-2 with that of other ethylenimine-derived compounds suggests

TABLE 3. Antiphage Spectrum of Ethylenimines (acetate buffer at pH 5.0, exposure 30 min)

Preparations	Concentration in moles	Type of bacteriophage			
		T1	T2	T6	T7
Ethylenimine	$1 \cdot 30^{-2}$	0,04	50	73	0,0004
	$1 \cdot 10^{-3}$	80	74	86	3,4
Ethoxen	$1 \cdot 10^{-2}$	0	40	33	0
	$1 \cdot 10^{-3}$	0,001	75	50	0,003
MEI-2	$1 \cdot 10^{-2}$	0	0,0001	0	0
	$1 \cdot 10^{-3}$	0,04	0,1	0,05	0,001
Thiosphophamide	$1 \cdot 10^{-2}$	0	0	0	0
	$1 \cdot 10^{-3}$	2,1	0,01	0,03	0,3
Dipine	$1 \cdot 10^{-2}$	0	51	62	0,0005
	$1 \cdot 10^{-3}$	1,5	71	86	0,01

that this preparation should have a considerable antimitotic effect. The confirmation of our hypothesis would be a strong argument for the utilization of the bacteriophage model for the selection of new potentially active antitumor preparations.

Thus, the antiphage activity of mono- and polyfunctional ethylenimine-derived compounds studied can be correlated with their antimitotic properties. The high biological activity of certain so-called monofunctional ethylenimines, ethoxen in particular, may be related to the presence in its molecule of several reaction groups, which form inter- or intra-molecular cross bonds in the biological macromolecule.

SUMMARY

Experimental data presented in this paper deal with the comparative study of the antiphage activity of ethylenimine and its mono- and polyderivatives. As established the degree of the inhibitory effect of the substances studied depended on their concentration, the pH of the buffer solution, the time of action upon the bacteriophage, and in some cases upon the type of bacteriophage. In the authors opinion, different antiphage activity of ethylenimine derivatives is completely comparable with their different anti-tumour effect. High biological activity of such monofunctional ethylenimines, as ethoxen (1-ethylenimino-2-oxibutan-3) may be connected with the presence in its molecule of several reaction-capable groups, forming cross bonds in the biological macromolecule not only at the expense of ethylenimine ring, but also of the unsaturated ethylene or hydroxyl group.

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