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EFFECT OF 8-METHOXYPSORALEN AND 4,5'-DIMETHYLANGELICIN ON RIBONUCLEIC ACID SYNTHESIS IN VITRO

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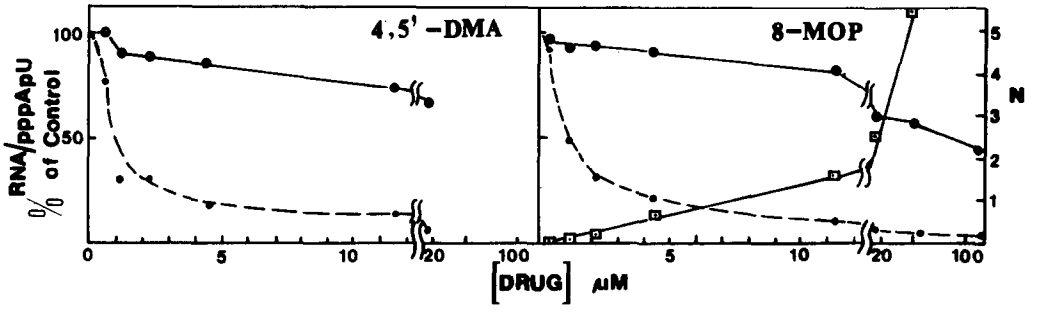
Photobinding of 8-methoxypsoralen (8-MOP) to DNA leads to mono- and diadduct formation while 4,5'-dimethylangelicin (4,5'-DMA) binds monofunctionally (1). The effect of the irreversible binding of furocoumarins to DNA on different steps of RNA synthesis has been assayed in this study.

The complexes were formed by irradiation of phage T7 DNA (250 μg/ml) in the presence of 8-MOP or 4,5'-DMA at the concentration indicated and purified by a gentle extraction of the non-covalently bound drug (2). The stoichiometry of the 8-MOP-DNA complexes was estimated using ^{3}H-labeled drug (2). DNA transcriptional template activity (an overall RNA synthesis) was assayed with E. coli RNA polymerase as described before (3). Synthesis of pppApU was assayed according to Johnston and Mc Clure (4) but ^{14}C-labeled UTP was used.

Figure 1. Inhibition of overall RNA synthesis by covalent adducts of 8-MOP and 4,5'-DMA and effect of the drugs on the formation of the initiating dinucleotide, pppApU



The complexes were formed by irradiation of DNA at the indicated drug concentrations and assayed for overall RNA (3) and pppApU syntheses. The amounts of RNA or dinucleotide were calculated as percentages of the corresponding controls. The number of 8-MOP molecules bound per 10^{3} DNA nucleotides (N- see right-hand scale) was estimated (2).

As shown in several laboratories (e.g. see ref. 5) covalent binding of furocoumarins to DNA considerably affects RNA synthesis catalysed by E. coli RNA polymerase (Figure 1). The most surprising observation is that the complexes of DNA with 4,5'-DMA show similar decrease of the amount of RNA synthesized to those of DNA with 8-MOP formed under identical conditions (see also Table 1). Although no stoichiometry of 4,5'-DMA-DNA complexes was estimated in our study it may be assumed that the overall adduct density does not differ much from that of 8-MOP-DNA (1). As however monoadducts only are formed with 4,5'-DMA while cross-links are induced by 8-MOP the observation presented here leads to a conclusion that the mono- and diadducts may be similarly toxic for the

template activity of phage T7 DNA. It has been recently reported (6) that both monoadducts and cross-links introduced to SV 40 DNA by photoreaction with 4'-hydroxymethyl-4,5',8-trimethylpsoralen induce termination of polynucleotide synthesis two bases away from the covalent adduct. This observation indicates that mechanisms of the inhibition of RNA synthesis by cross-links and monoadducts, if the latter are bound to the coding strand, are similar.

Table 1. Template activity of 8-MOP- and 4,5'-DMA-DNA complexes under nonreinitiating and reinitiating conditions

Drug concentration (μM)	8-MOP		4,5'-DMA	
	Assay conditions			
	non-reinitiating	reinitiating	non-reinitiating	reinitiating
0	100%	100%	100%	100%
0.6	99.8±8.8%	83.4±6.4%	78.9±2.0%	63.5±5.2%
2.3	60.4±7.1%	43.5±5.1%	69.7±1.3%	38.9±4.7%
11.5	26.6±3.7%	9.0±1.9%	34.6±0.1%	11.0±3.6%
19.0	18.7±4.3%	5.6±0.1%	28.0±1.3%	9.5±3.2%

DNA (250 μg/ml) irradiated in the presence of the indicated drug concentration and purified was preincubated for 15 min. at 37° with RNA polymerase, ATP, GTP and [^{14}C]UTP then either $(NH_4)_2SO_4$ to 0.4 M and CTP (non-reinitiating conditions) or KCl to 0.1 M and CTP were added (conditions allowing reinitiation). The samples were incubated for 20 min. (7). Template activity is expressed as percentage of the corresponding controls.

The binding of the enzyme to 8-MOP-DNA, the stability of the complexes of RNA polymerase with modified DNA were not appreciably affected when compared with native DNA or irradiated DNA (not shown). The two drugs inhibit to some extent initiation as inferred from decrease of pppApU synthesis on the furocoumarin-DNA complexes. The amount of the initiating dinucleotide synthesized on DNA containing 2.5 molecules of 8-MOP per 10^3 nucleotides decreased to 63% while the overall RNA synthesis was inhibited to a few percent. 4,5'-DMA exhibited similar effect on abortive initiation (Figure 1). Elongation step is affected by 8-MOP depending on the adduct density. When DNA containing 6.2 drug molecules per 10^3 nucleotides is used as a template a considerable amount of the synthesized RNA is eluted in the peak which follows a marker tRNA on Sepharose 4B column (not shown). In order to check whether the drugs inhibit release of the enzyme from the template, RNA synthesis was assayed on DNA either under non-reinitiating conditions or under conditions which allow reinitiation (Table 1). Higher inhibitory effects were observed with both drugs when reinitiation was possible. These results suggest that the enzyme remains attached to the template after encountering an adduct molecule and the effect is similar in both cases.

It is concluded from this study that 8-MOP blocks movement of RNA polymerase along the template, inducing premature termination and inhibits recycling of the enzyme. In some experimental systems the monofunctionally binding furocoumarin, 4,5'-DMA shows similar characteristics.

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