A C T A U N I V E R S I T A T I S L O D Z I E N S I S
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4',6-DIAMIDINE-2-PHENYLINDOLE (DAPI) PREFERENTIALLY INHIBITS POLY A SYNTHESIS CATALYSED BY BACTERIAL RNA POLYMERASE*

4',6-Diamidine-2-phenylindole (DAPI), a dye interacting preferentially with dA : dT sequences of DNA and showing high affinity to the hybrid poly rA : poly dT exhibits a stronger inhibitory effect on poly A than on RNA synthesis in vitro catalysed by Escherichia coli DNA-dependent RNA polymerase.

4',6-Diamidine-2-phenylindole (DAPI) forms strong complexes with DNA and inhibits DNA-dependent DNA and RNA synthesis in vitro [2, 10-12, 14]. Experimental data have led to different models concerning the structure of the complex. Intercalative [2, 3, 10-12] or groove - binding of the drug to DNA [9] has been postulated. Nevertheless, high preference to dA : dT sequences of DNA is generally acknowledged. Some data indicate that the drug exhibits high affinity to the hybrid poly rA : poly dT [2]. The high inhibitory effect of DAPI on enzymatic reactions involving this type of hybrid has been predicted by Mildner et al. [13]. Escherichia coli DNA-dependent RNA polymerase (ribonucleoside triphosphate : RNA nucleotidyltransferase, E.C. 2.7.7.6) incubated with ATP synthesizes poly A on short oligothymidylic sequences of DNA [4, 5] Hence, the reaction provides an interesting model to assay the drug specificity.

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MATERIALS AND METHODS

DAPI was synthesized by Dr J. Kapuściński. The other materials and E. coli RNA polymerase were the same as previously described [6, 8]. Reaction mixtures as specified [6] contained either [^{14}C] ATP as a sole substrate (poly A synthesis) or [^{14}C]ATP with the other three unlabelled nucleoside triphosphates (RNA synthesis), KCl at the concentration indicated, 2 mM Mn^{2+}, 8 µg of native calf thymus DNA and about 1 Burgess' unit of the enzyme per assay volume (0.25 ml) were used. The other assay conditions were as described before [6]. Polynucleotide synthesis in the presence of inhibitors was assayed at two or three different drug concentrations and expressed in percent of the corresponding controls (i.e assays without an inhibitor). Drug concentrations decreasing the synthesis to 50% were read from inhibition curves.

RESULTS AND DISCUSSION

Relative rates of RNA and poly A syntheses and inhibitory effects of DNA-interacting ligands depend on the ionic strength and the activator used [15]. In the presented experiments the effect of DAPI is assayed in the presence of native DNA and Mn^{2+}. The yields of the two types of the formed product are comparable under these conditions [15]. Concentration of DAPI inhibiting heteropolymer synthesis to 50% decreases from 35.4 µM in the absence of KCl to 13.6 µM at 0.15 M KCl it does not practically vary for the poly A synthesis (Tab. 1). Concentrations of the drug decre-

T a b l e 1

Concentration of DAPI (µM) decreasing RNA and poly A synthesis to 50%
Stężenie DAPI (µM) hamujące syntezę RNA i poly A do 50%

KCl (M)	RNA	Poly A
0.00	34.5 ± 8.8 (4)	5.5 ± 1.3 (3)
0.05	30.0 ± 8.5 (4)	6.6 ± 1.8 (5)
0.15	13.6 ± 1.4 (4)	5.3 ± 1.5 (4)

N o t e: The averages of three-five independent experiments (as indicated in the parantheses) ± standard deviations are shown.

asing RNA and the homopolymer formation differ by factor of 6.5, 4.5 and 2.5 at 0.0, 0.05 and 0.15 M KCl, respectively. Other ligands (ethidium bromide, acriflavin, distamycin A and netropsin) show either no differences or lower differences. At most twice lower concentration of distamycin has been needed for poly A synthesis than RNA synthesis to decrease their rate to 50% [15]. Similarly the higher inhibitory effect of DAPI on poly A synthesis was found using denatured DNA as a template (not shown).

As shown by C h a m b e r l i n and B e r g [4, 5] poly A chains are synthesized with ATP by repetitive transcription of oligothymidylic sequences in DNA in the absence of other nucleoside triphosphates. rA : dT structures should be transiently formed then and the course of the reaction is dependent on their formation and separation allowing a reuse of the template fragment by the enzyme and on the elongation of the synthesized chains. The higher inhibitory effect of DAPI on poly A than on RNA formation may be due to a preferential binding of the drug to these rA : dT structures which increases the stability of the product - template complex, hence decreasing a rate of the synthesis. Alternatively, DAPI may inhibit poly A synthesis by interaction with oligo dA : dT sequences of DNA preventing their use in poly A synthesis. The latter mode of interaction seems to be less important for the effect recorded here as poly A synthesis occurs primarily on single stranded regions of DNA [5]. Considerable differences in inhibition of RNA and poly A synthesis are consistent with the postulated high affinity of the drug to dT : rA hybrid structures [2].

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PREFERENCYJNE HAMOWANIE SYNTEZY POLI A KATALIZOWANE BAKTERYJNĄ POLIMERAZĄ RNA PRZEZ 4',6-DIAMIDYNO-2-FENYLOINDOL (DAPI)

4',6-Diamidyno-2-fenyloindol (DAPI), związek wykazujący preferencję w stosunku do sekwencji typu dA : dT i wysokie powinowactwo do hybrydu poli rA : : poli dT hamuje w znacznym stopniu syntezę poli A niż syntezę RNA katalizowaną in vitro przez zależną od DNA polimerazę RNA E. coli.