CIBACRON BLUE INHIBITION OF PROKARYOTIC AND EUKARYOTIC DNA-DEPENDENT RNA POLYMERASES

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A comparison was drawn between the action of Cibacron Blue F3GA on the enzymic activity of DNA-dependent RNA polymerases from different sources, e.g. *Escherichia coli*, calf thymus and wheat germ (polymerase II). Sensitivity towards this inhibitor was determined for polymer formation and primed abortive synthesis of trinucleotide UpApU. In case of *E. coli* polymerase and wheat germ polymerase II the dye inhibits both polymer formation and abortive synthesis. Calf thymus polymerase II is inhibited only in the polymerisation step. The primed initiation reaction was found to be resistant towards the dye. In case of *E. coli* polymerase and wheat germ polymerase II the sensitive step is the formation of internucleotide bond whereas in case of calf thymus polymerase II the translocation of the enzyme is influenced. An analysis of kinetic data indicates more than one binding site for the dye on RNA polymerase II from calf thymus and wheat germ. Cibacron blue does not inhibit specific transcription catalyzed by RNA polymerase III from human HeLa cells and mouse leukemia L1210 cells.

Nuclear genes in eukaryotic cells are transcribed by three DNA-dependent RNA polymerases designated RNA polymerase I, II and III (see review[1-3]). These enzymes involve in analogy to their prokaryotic counterpart several subunits. Recently, a partial amino acid sequence homology in the largest subunits of the prokarytoic and eukaryotic enzyme suggesting their common origin was shown[4-7]. The eukaryotic transcription apparatus is apparently more adequately described concerning the function of essential transcription protein factors than concerning the catalytical properties and the role of individual subunits of nuclear RNA polymerases (for a recent review see ref.[8]). For this reason specific inhibitors are valuable tools to investigate transcription especially by studying the catalytic mechanism and the role of active sites of RNA polymerases. Nevertheless, up to now inhibitors of eukaryotic RNA polymerases were used much less for this purpose in comparison with prokaryotic enzymes (for review see refs[9,10]). However, recent observations demonstrate the validity of this approach for the study of initiation of transcription by eukaryotic RNA polymerases especially for the elucidation of the assembly of transcription initiation complexes[11-15].

One of the inhibitors successfully used is α-amanitin, a bicyclic octapeptide, which exhibits a different inhibitory activity towards eukaryotic RNA polymerases[3]. Recently, detailed analysis has shown that α-amanitin inhibits translocation of RNA polymerase II after the formation of the first internucleotide bond[16]. Another inhibitor is Cibacron Blue F3GA, a sulfonated polyaromatic dye, which has been shown to inhibit prokaryotic RNA polymerase of *Azotobacter vinelandii*[17] as well as yeast RNA polymerase I (ref.[18]). Whereas a noncompetitive mechanism with respect to substrates and DNA was found for prokaryotic enzyme, uncompetitive mechanism was described for yeast RNA polymerase I.

In this paper the inhibitory effect of Cibacron Blue F3GA on the in vitro transcription both by RNA polymerase II (calf thymus, wheat germ) and RNA polymerase III (human HeLa and mouse leukemia L1210 cells) was studied and its mechanism of action proposed. To compare these results with bacterial systems the inhibitory effect of the dye on *E. coli* RNA polymerase was followed.

EXPERIMENTAL

Reagents. ATP and UTP (HPLC purified), creatine kinase from rabbit muscle, creatine phosphate Na_2-salt, Cibacron Blue F3GA as well as acrylamide gels components were purchased from Serva (F.R.G.). Aqueous solutions of required concentrations of Cibacron Blue were prepared using an extinction coefficient of 13·6 $mmol^{-1}$ cm^{-1} at 610 nm (ref.[17]) and stored at −20°C; [α-^{32}P] UTP (s.a. 15·17 TBq/mmol) was from Amersham (England). Poly[d(A−T)] (Boehringer, Austria) was dissolved in 0·15M-NaCl and stored at −20°C; plasmid pVA was a gift from Dr I. Grummt (Würzburg, F.R.G.) purified according to a standard protocol using double cesium chloride gradient centrifugation. DEAE cellulose (DE-52) and phosphocellulose (P 11) were from Whatman (England). Biogel A-1·5 m was purchased from Biorad (U.S.A.). UpA (HPLC purified) was prepared in this Institute by Dr Holý. X-ray films were from Fotochema (Czechoslovakia). All buffer components were reagent grade; redistilled water was used.

RNA polymerase from *E. coli* was prepared from strain K12 by the method of Burgess and Jendrisak[19]; DNA-cellulose chromatography step was followed by further enzyme purification using phosphocellulose column according to Gonzales et al.[20]. Holoenzyme was found to be ATPase free[21] and its purity was 98% as judged by scanning of SDS-polyacrylamide gel. Specific activity of the enzyme used was 20 500 units/mg with T7 DNA as template according to the standard assay[20]. The enzyme was stored at −20°C.

RNA polymerase II from wheat germ was purified according to Jendrisak and Burgess[22] using DEAE cellulose and phosphocellulose chromatography with several modifications[23]. Specific activity of the enzyme was 110 units/mg according to standard assay[22]. The enzyme was stored in liquid nitrogen. RNA polymerase II from calf thymus was purified according to Hodo and Blatti[24]. The phosphocellulose pooled peak had a specific activity 11 units/mg according to standard assay[24]. The enzyme was stored at −80°C.

Nuclear extracts containing RNA polymerase III activity were prepared from HeLa cells and L1210 cells according to Dignam et al.[25]and Haglund and Rothblum[26], respectively.

RNA *synthesis catalyzed by E. coli* RNA *polymerase.* The standard reaction mixture (50 μl) contained buffer A (40 mM Tris–HCl, pH 7·9, 80 mM KCl, 10 mM $MgCl_2$ and 1 mM dithiothreitol), 100 μM ATP, 10 μM [α-^{32}P] UTP (2·3 kBq), 0·5 μg of poly[d(A−T)] and 2 μg of enzyme pro-

tein[27]. The concentration of Cibacron Blue varied in the range 0—10 μmol l^{-1}. In kinetic experiments the concentration of ATP varied from 10 to 100 μmol l^{-1} and the concentration of inhibitor was 2, 4 and 6 μmol l^{-1}. After a 10 min incubation period at 37°C the reaction mixtures were cooled in ice cold water and precipitated with trichloroacetic acid as described below. Some reaction mixtures (containing 74 kBq [α-^{32}P]UTP per assay) were analyzed using 7M urea/24% polyacrylamide gels as described below.

Synthesis of UpApU *catalyzed by E. coli* RNA *polymerase.* The standard reaction mixture (15 μl) contained buffer A, 100 μM UpA, 10 μM [α-^{32}P] UTP (2·3 kBq), 0·025 μg poly [d(A—T)] and 1·2 μg of enzyme protein[28]. The concentration of Cibacron Blue varied from 1 to 10 μmol . . l^{-1}. The concentration of UpA as well as of the dye for the determination of kinetic constants is mentioned in the legend to Fig. 2. The reaction mixtures were incubated for 10 min at 37°C, the reaction was terminated by adding EDTA to a final concentration of 80 mmol l^{-1} and mixtures were analyzed by paper electrophoresis as described below.

RNA *synthesis catalyzed by wheat germ* RNA *polymerase* II. The standard reaction mixture (50 μl) contained buffer B (40 mM Tris-HCl, pH 7·9, 60 mM KCl, 1 mM $MnCl_2$, 0·5 mM dithiothreitol, 0·04 mM EDTA and 8% glycerol), 250 μM UpA, 100 μM ATP, 10 μM [α-^{32}P] UTP (2·3 kBq), 0·5 μg poly d(A—T) and 0·9 μg of enzyme protein[23]. For the concentration of ATP and inhibitor used in the kinetic studies see legend to Fig. 4*a*. After a 10 min incubation period at 37°C the radioactivity of the acid precipitable material was determined as described below.

Synthesis of UpApU *catalyzed by wheat germ* RNA *polymerase* II. The reaction mixture (15 μl) contained buffer B, 100 μM UpA, 10 μM [α-^{32}P] UTP (2·3 kBq), 0·3 μg poly [d(A—T)] and 1 μg of enzyme protein[23]. The concentration of Cibacron Blue varied from 1—16 μmol l^{-1}. The concentration of UpA as well as of the dye used for the determination of kinetic constants are given in the legend to Fig. 4*b*. The reaction mixtures were incubated for 30 min at 37°C, the reaction was terminated by adding EDTA to a final concentration of 80 mmol l^{-1} and mixtures were analyzed by paper electrophoresis (see below).

RNA *synthesis catalyzed by calf thymus* RNA *polymerase* II. The standard reaction mixture (15 μl) contained buffer C (50 mM Tris-HCl, pH 7·9, 1·7 mM $MnCl_2$, 1·25 mM $MgCl_2$, 100 mM ammonium sulphate, 0·125 mM dithiothreitol, 0·025 mM EDTA and 8% glycerol), 600 μM creatine phosphate, 4·5 μg creatine kinase, 600 μM ATP, 10 μM [α-^{32}P] UTP (2·3 kBq), 1·25 μg poly [d(A—T)] and 2·75 μg of enzyme protein[24]. The concentration of Cibacron Blue varied from 1—10 μmol l^{-1}. The concentration of the inhibitor and ATP used for the kinetic measurements are given in the legend to Fig. 6. The reaction mixtures were incubated for 15 min at 37°C, the reaction was terminated by adding EDTA and mixtures were analyzed by paper electrophoresis as described below.

Synthesis of UpApU *catalyzed by calf thymus* RNA *polymerase* II. The composition of the reaction mixture (15 μl) was the same as in the case of RNA synthesis catalyzed by calf thymus RNA polymerase II. The concentration of Cibacron Blue varied from 1—10 μmol l^{-1}. The reaction mixtures were incubated for 90 min at 37°C, the reaction was terminated by adding EDTA and mixtures were analyzed by paper electrophoresis as described below.

Purification of ternary transcription complexes. E. coli RNA polymerase was incubated (in a final volume of 15 μl) in buffer A with 2 μg poly [d(A—T)] and 4 μg of enzyme protein for 10 min at 37°C. Thereafter ATP and [α-^{32}P]UTP (185 kBq) to a final concentration of 50 and 5 μM, respectively, were added. After a 5 min incubation period at 37°C the reaction mixture was loaded at room temperature onto a Biogel A 1·5 m column (7 × 45 mm) equilibrated with buffer D (40 mM Tris-HCl, pH 7·9, 7 mM $MgCl_2$, 80 mM KCl, 1 mM dithiothreitol and 5% glycerol)

and eluted from the column with the same buffer. Fractions (3 drops) were collected and counted according to Cerenkov. The ternary transcription complex consisting of enzyme, template and nascent RNA was eluted immediately after void volume of the column and was stored for several days at 4°C.

Effect of Cibacron Blue on the activity of transcription complexes. Transcription ternary complex (10 μl) was incubated (final volume 50 μl) in buffer D with 100 μM ATP and 10 μM [α-^{32}P] UTP (1·6 kBq) with/without Cibacron Blue (8 μmol l^{-1}) for 30 min at 37°C. Synthesized RNA was precipitated with trichloroacetic acid and analyzed as described below. In control experiment the ternary transcription complex was incubated without substrates.

Synthesis of virus-associated RNA *catalyzed by* RNA *polymerase* III. The standard reaction mixture (25 μl) contained 20 mM Tris–HCl, pH 7·9, 7·5 mM $MgCl_2$, 75 mM KCl, 0·5 mM dithiothreitol, 0·1 mM EDTA, 12% glycerol, 0·25 mM phenylmethylsulphonyl fluoride (PMSF), 600 μM each of ATP, CTP and GTP, 25 μM [α-^{32}P] UTP (185 kBq), 2 μg pVA DNA and 1 μg/ml of α-amanitin. The reaction was started by adding of 12·5 μl of nuclear extract and incubated for 90 min at 30°C (ref.[29]). The reaction was terminated by adding 175 μl of stop solution (50 mM sodium acetate, pH 5·5, 0·5% sodium dodecylsulfate and 50 μg/ml of tRNA); extraction with saturated phenol as well as with chloroform was carried out. Ammonium acetate solution (5M) was added to the aqueous phase to a final concentration of 0·2M and RNA was then precipitated overnight with two volumes of ethanol at −20°C. The sediment of RNA was washed with 80% ethanol, lyophilized, dissolved in 20 μl of deionized formamide, denatured at 65°C for 5 min and analyzed using 7M urea/6% acrylamide gel electrophoresis as described below.

Analysis of reaction products. a) Precipitation of RNA by trichloroacetic acid: After incubation 5 μl of bovine serum albumin (7·5 mg/ml) were added as a carrier, followed by 0·5 ml of 5% trichloroacetic acid with 1% of sodium pyrophosphate. After standing for 30 min in ice cold water the acid precipitable material was collected on Whatman GF/C fibre filters and washed successively with 2·5 ml each of 5% and 0·5% trichloroacetic acid and finally with 2·5 ml of ethanol. The radioactivity on dried filters was measured in PPO/POPOP scintillation cocktail. *b*) Analysis on polyacrylamide gels was performed under denaturating conditions according to Maxam and Gilbert[30]; after autoradiography the radioactive spots were cut out and counted according to Cerenkov. *c*) Analysis by paper electrophoresis: The reaction mixtures were loaded on Whatman 3 MM paper and analyzed in 0·05M sodium dihydrogen citrate, pH 3·8 at 1 000 V for 60 min. Electrophoreograms were autoradiographed overnight at −70°C using an intensifying screen (Perlux, VEB Kali Chemie, G.D.R.), radioactive strips wre cut out and counted in a toluene scintillation cocktail.

Curve fitting procedure: Kinetic data were computed using Enzfitter software (written by R. J. Leatherbarrow, Elsevier, Cambridge 1987).

RESULTS

In this paper we have investigated the effect of Cibacron Blue on overall RNA synthesis as well as on the abortive production of the trinucleotide UpApU using RNA polymerases II purified from calf thymus and wheat germ and RNA polymerase from *E. coli*. To obtain consistent data for RNA polymerases originating from different sources the synthetic template poly[d(A−T)] was used in all transcription assays. RNA polymerase III activity present in nuclear extracts isolated from HeLa

and mouse leukemia K1210 cells was tested using transcription units for virus-associated RNAs (VAI and VAII) from the adenovirus genome as a template.

Effect of Cibacron Blue on E. coli RNA *Polymerase*

The effect of increasing concentration of the dye (up to 12 μmol l^{-1} final concentration) on the synthesis of RNA in the form of poly[r(A−U)] polymer and the trinucleotide UpApU was determined. Figure 1 shows that the synthesis of RNA is more inhibited than the formation of the abortive oligonucleotide UpApU. The 50% inhibition of the polymer synthesis was observed at about 1 μmol l^{-1} concentration of inhibitor, i.e. at a lower value than that described for *A. vineladii* RNA polymerase[17]. The different degree of inhibition by Cibacron Blue in these two prokaryotic systems is still more pronounced for UpApU synthesis. A 50% inhibition of UpApU synthesis was found at the 3·2 μmol l^{-1} concentration of inhibitor. A complete inhibition was observed at a concentration of 8 μmol l^{-1} of the dye whereas in the case of *A. vinelandii* RNA polymerase 52% of its activity is preserved at the same concentration of the inhibitor[17].

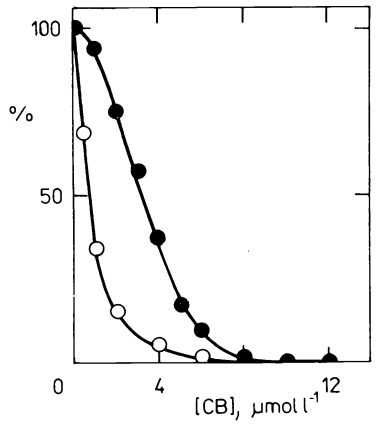

FIG. 1

Inhibition of *E. coli* RNA polymerase by Cibacron Blue (CB), % relative enzyme activity. ○ Synthesis of RNA, ● synthesis of UpApU

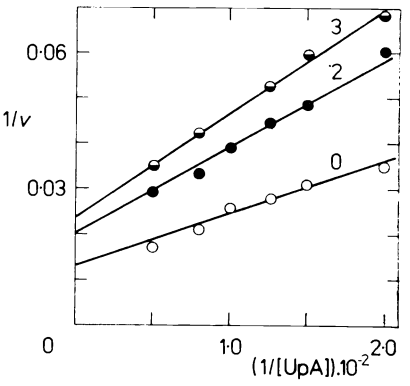

FIG. 2

Initial velocity pattern for UpA primed UpApU synthesis catalyzed by *E. coli* RNA polymerase with/without Cibacron Blue. Concentration of UTP was 10 μmol l^{-1}, concentrations of inhibitor (Cibacron Blue) in μmol l^{-1} are given in the figure, concentration of UpA (ordinate) is given in μmol l^{-1}

Inhibition by Cibacron Blue was also studied at varying concentrations of ATP, poly[d(A–T)] and UpA. Figure 2 shows that this dye is a noncompetitive inhibitor with respect to the binding of UpA to RNA polymerase. An analogous mechanism was found with varying concentrations of ATP and template (data not shown). These observations are in accordance with data described by Kumar and Krakow[17]. This mechanism implies that binding of Cibacron Blue does not affect the binding of substrates. Our observation that the sensitivity of UpApU formation to Cibacron Blue is greater than previously shown[17] suggests that the formation of the internucleotide bond is affected by the inhibitor.

To determine the effect of Cibacron Blue during transcription we have isolated the active transcription complex using gel filtration of the stable complex. At 8 μmol l^{-1} concentration of the inhibitor when RNA synthesis is completely blocked (Fig. 1) we have found only insignificant inhibition indicating the resistance of the transcription complex towards the dye. The observed inhibition may be caused by a small population of active enzymes molecules entering a next round of transcription cycle.

Effect of Cibacron Blue on Wheat Germ RNA *Polymerase* II

The inhibition by Cibacron Blue of RNA and UpApU synthesis catalyzed by RNA polymerase II purified from wheat germ was investigated (Fig. 3). Whereas the 50% inhibition of RNA synthesis is reached at a concentration of 5 μmol l^{-1} of the dye, the synthesis of UpApU is more sensitive since the same degree of inhibition is observed already at a concentration of 2 μmol l^{-1} of the inhibitor. The shape of the inhibition curve for RNA synthesis shows additional effects of the dye with increasing concentration (Fig. 3). A similar but more pronounced effect was found for calf thymus RNA polymerase II and its possible reason is discussed below.

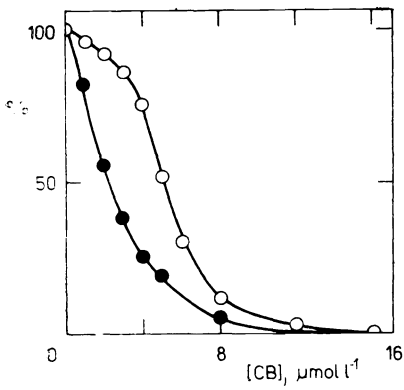

FIG. 3

Inhibition of wheat germ RNA polymerase II by Cibacron Blue (CB), % relative enzyme activity. ○ Synthesis of RNA, ● synthesis of UpApU

Double reciprocal plot of $1/v$ versus $1/[ATP]$ for RNA synthesis is in agreement with the uncompetitive mechanism in the selected range of inhibitor concentrations (Fig. 4*a*). An analogous plot $1/v$ versus $1/[UpA]$ for UpApU formation (Fig. 4*b*) suggests the same inhibition mechanism. Since both the polymerase reaction and the first internucleotide bond formation are affected we conclude that it is the internucleotide bond formation which is inhibited by Cibacron Blue.

Effect of Cibacron Blue on Calf Thymus RNA *Polymerase* II

The inhibition curves for mammalian RNA polymerase II were obtained in a similar way as mentioned above (Fig. 5). Although RNA synthesis is inhibited in the range up to 8 μmol l^{-1}, UpApU formation is only weakly sensitive. In the case of UpApU synthesis we have observed the reproducible maximum at a concentration of 3 μmol . . l^{-1} of the inhibitor; this finding is consistent with a mild stimulatory effect observed also for other enzymes[31]. The shape of the RNA inhibition curve has a biphasic character which indicates a complicated mechanism of action of the inhibitor depending on its concentration.

The type of inhibition caused by this dye was determined with respect to the concentration of ATP. The Lineweaver–Burk plot is shown on Fig. 6 and analyzed according to Segel[32]. In order to distinguish between several mechanisms we plotted K_i (slope) versus [i] (see left insert in Fig. 6) and constructed a Dixon plot (see right insert in Fig. 6). Nonlinear replots indicate that the K_i (slope) term contains

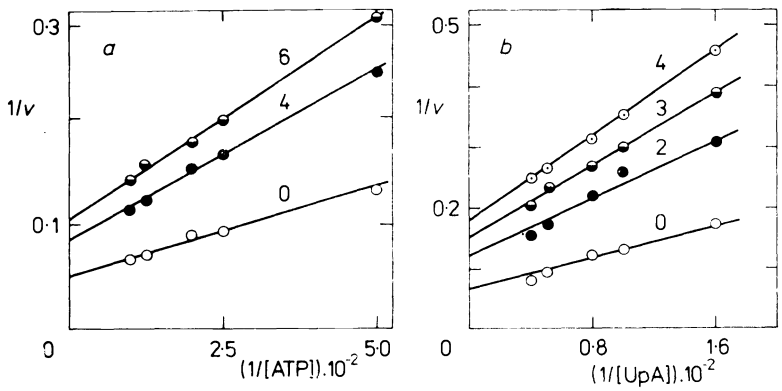

FIG. 4

Initial velocity pattern for RNA synthesis (*a*) and UpA primed UpApU synthesis (*b*) catalyzed by wheat germ RNA polymerase II with/without Cibacron Blue. Concentration of UTP was 10 μmol l^{-1}, concentrations of inhibitor (Cibacron Blue) in μmol l^{-1} are given in the figure, concentrations of ATP and UpA (ordinates) are given in μmol l^{-1}

higher powers of concentration of the inhibitor, or, in other words, that there are more than two inhibitor binding sites[32].

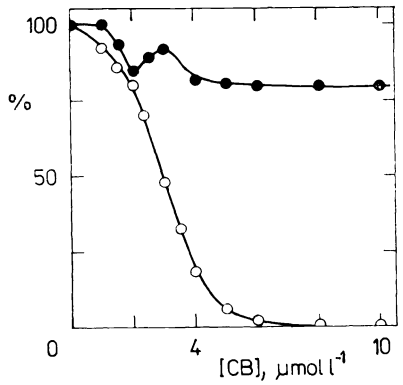

FIG. 5

Inhibiton of calf thymus RNA polymerase II by Cibacron Blue (CB), % relative enzyme activity. ○ Synthesis of RNA, ● synthesis of UpApU

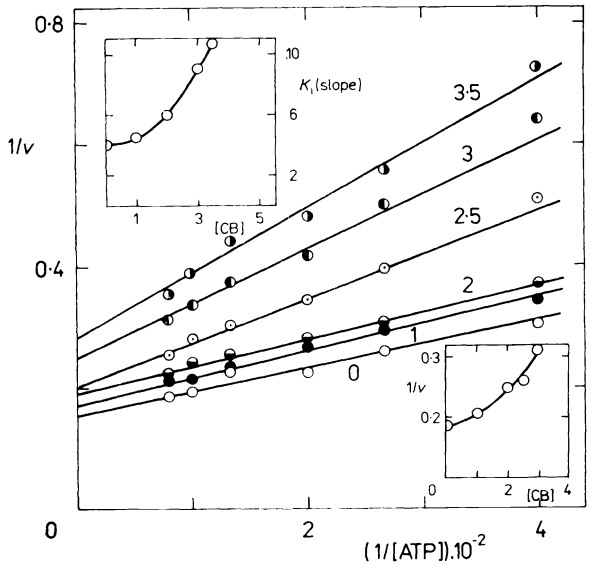

FIG. 6

Initial velocity pattern for RNA synthesis catalyzed by calf thymus RNA polymerase II with/ /without Cibacron Blue. Concentration of UTP was 10 μmol l^{-1}, concentrations of inhibitor (Cibacron Blue) in μmol l^{-1} are given in the figure. Left insert: plot K_i (slope) versus concetration of inhibitor (ref.[32]) for 100 μmol l^{-1} ATP. Right insert: Dixon plot $1/v$ versus concentration of inhibitor for 100 μmol l^{-1} ATP

The most straightforward explanation of the inhibitory mechanism in case of calf thymus RNA polymerase II is that Cibacron Blue inhibits translocation of the enzyme along the template since the first internucleotide bond formation is almost insensitive towards the dye.

Effect of Cibacron Blue on RNA *Polymerase* III

The effect of Cibacron Blue at a concentration up to 20 μmol l^{-1} on the transcription system of RNA polymerase III was investigated using human RNA polymerase III present in nuclear extracts from HeLa cells. The reactions were performed in presence of 1 μg/ml of α-amanitin to inhibit RNA polymerase III activity. The used template has two similar transcriptions units for RNA polymerase II which direct the synthesis of RNAs having the length 150 and 165 nucleotides. An analysis of autoradiograms has shown no inhibition effect of the dye for RNA polymerase III present in extracts from HeLa cells. Similarly in case of RNA polymerase III present in nuclear extracts from mouse leukemia L1210 cells we have found a resistance towards Cibacron Blue.

DISCUSSION

Several concepts on interaction of Cibacron Blue F3GA with various enzymes and proteins were suggested. According to Thompson and Stellwagen[33,34] the dye binds to a supersecondary structure known as "dinucleotide fold" by combination of hydrophobic and ionic interactions which occur in different proteins. In case of RNA polymerase this binding region may be present as a part of the template binding domain, substrate binding sites and product binding site. The complexity of the RNA polymerase molecule does not exclude other sites. The second supposes that the binding of the dye occurs nonspecifically to hydrophobic pockets on the protein[35,36]. However, the exact nature of its binding sites has not been elucidated up to now although different enzymes and proteins are currently purified using affinity columns with Cibacron Blue as ligand (Blue Sepharose from Pharmacia, Affi-Gel Blue gel from Bio-Rad).

This paper demonstrates that Cibacron Blue inhibits all reactions tested with *E. coli* RNA polymerase and wheat germ RNA polymerase II. In case of calf thymus RNA polymerase II the dye inhibits only the polymerisation reaction on poly [d(A−T)] template and calf thymus DNA (our unpublished results). The primed initiation reaction using UpA and UTP, however, was not inhibited. The specific transcription of VA genes catalyzed by RNA polymerase III was not blocked by this compound at all.

Since the data obtained by acid precipitation of the reaction products do not show the relative representation of all reaction products the reaction mixtures were ana-

lyzed using urea/polyacrylamide gels. No changes in the relative representation of the reaction products concerning the length and amount of oligonucleotides produced during initiation were found (L. Skálová, unpublished results).

It is of interest to compare our results concerning the effect of Cibacron Blue on eukaryotic RNA polymerase II and III with results published on yeast RNA polymerase I where uncompetitive inhibition with respect to concentration of ATP and DNA were found[18]. Our results suggest a noncompetitive mechanism of inhibition of plant polymerase II and a complex interaction with calf thymus polymerase II. This spectrum of different mechanisms both from the point of view of transcription cycle and interactions with substrates and DNA is not surprising since there may exist various types and a number of binding sites for this dye at different polymerases. This observation offers further perspectives to use Cibacron Blue as a probe for spectroscopical analysis of this interaction.

Recent observation that eukaryotic RNA polymerase II can be purified using a column with Cibacron Blue as ligand requires special attention[37]. In this case Cibacron Blue may act as a tightly bound inhibitor. Thus the formation of enzyme–dye complex and enzyme–dye–substrate complex eliminate substantially the concentration of free inhibitor and therefore simple methods of determining K_i cannot be applied. The analysis according to Dixon[38] shows that this indeed is valid for wheat germ RNA polymerase II (Fig. 7). Similar result may be found for calf thymus RNA polymerase II and *E. coli* enzyme as well. Therefore we do not use the term

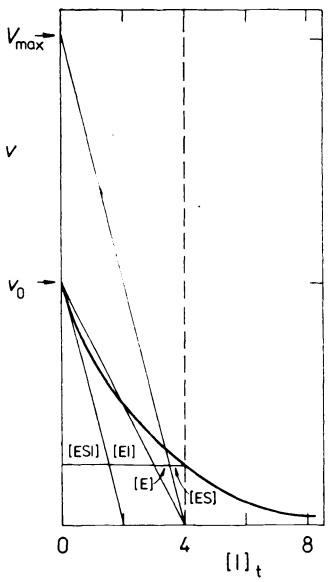

FIG. 7

Plot suggested by Dixon[39] to determine distribution of enzyme species at 4 μmol l^{-1} Cibacron Blue in case of noncompetitive inhibition for UpApU synthesis catalyzed by wheat germ RNA polymerase II. ESI enzyme–substrate–inhibitor complex; EI enzyme–inhibitor complex; E free enzyme; ES enzyme–substrate complex; $[I]_t$ concentration of the inhibitor; v reaction velocity in arbitrary units; v_0 initial velocity of reaction in absence of inhibitor; V_{max} maximal initial velocity in absence of inhibitor for 1/[UpA] limiting to zero; concentration of ATP (ordinate) is given in μmol l^{-1}

K_i which was used in the quoted papers[17,18]. Nevertheless, inhibition curves determined in this and other papers[17,18] can be directly compared.

Taken together the action of Cibacron Blue may be compared with action of the most typical inhibitor of eukaryotic RNA polymerase α-amanitin. The major difference consists in the fact that Cibacron Blue inhibits both eukaryotic and prokaryotic RNA polymerases. The kinetic analysis reported here shows that there is more than one binding site for the dye on RNA polymerase II which is in contrast to the existence of one strong binding site for α-amanitin[16,39,40]. Cibacron Blue mainly inhibits the formation of internucleotide bonds and in case of calf thymus RNA polymerase II it apparently inhibits the translocation of the enzyme along DNA.

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