Stimulation of transcription on chromatin by polar organic compounds.

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Received 12 February 1976

ABSTRACT

Polar organic compounds, including DMSO, increase RNA synthesis on isolated chromatin by E. coli RNA polymerase and RNA polymerase II from calf thymus. Transcription is stimulated on chromatin from Friend-virus-infected erythroleukemia cells and from various other sources. Using procedures which inhibit specifically the formation of a stable initiation complex, it is shown that the stimulation does not result from an increase in initiation of both E. coli and the eukaryotic RNA polymerase. After separation of chromatin into template active and inactive fractions, DMSO increases RNA synthesis by a factor of about 1.5 using the template inactive fraction, while stimulation of transcription on the template active portion is lower (factor of 1.2). It is suggested that the effect on RNA synthesis is mediated by a weakening of the apolar interactions between histones in chromatin subunits, releasing transcription partially from the constraints imposed by histones.

INTRODUCTION

Polar organic compounds can induce Friend-virus-infected erythroleukemia cells (Friend cells) to differentiate into hemoglobin synthesizing erythroid cells^{1,2}. Of these compounds, dimethylsulfoxide (DMSO) is the most extensively studied one². DMSO must reach an adequate intracellular concentration to initiate hemoglobin synthesis³. RNA sequences complementary to a DNA copy of mouse globin mRNA can be detected in Friend cells after 2 days of exposure to DMSO, but little or no hybridization is observed in uninduced cells⁴. This indicated that induction of erythroid differentiation is mediated by an alteration in the program of transcription. As gene expression in eukaryotes is closely related to chromatin conformation⁵, it may be

suggested that the induction of gene transcription is caused by an alteration in chromatin structure. This suggestion receives support from a current model of chromatin structure which envisages chromatin as organized discontinuously in subunits⁶⁻⁹. An essential feature in this model is the formation of a hydrophobic core made of the apolar histone regions⁹⁻¹¹. An alteration of the physicochemical properties of the medium can be expected to have some influence on the hydrophobic interactions in chromatin subunits, and thereby on the restrictions imposed by histones on DNA transcription. Physicochemical investigations¹² have indicated already a relaxation of chromatin structure by DMSO. The present study uses the activity of added RNA polymerases as a sensitive probe to measure the effect of polar organic compounds on the structure of isolated chromatin.

MATERIALS AND METHODS

Materials. All reagents were certified grade and purchased from Merck, Darmstadt. [³H]UTP (18 Ci/mmol) was from Buchler, Braunschweig. The preparation of *E. coli* RNA polymerase¹³ and RNA polymerase II from calf thymus¹⁴ have been described.

Cells. The Friend-virus-infected erythroleukemia cells (clone FSDI/F4) were kindly provided by Dr. R. Neth and cultured as described¹⁵.

Chromatin. Nuclei were purified according to Chevaillier and Philippe¹⁶. Chromatin fractionation by ECTHAM-cellulose chromatography¹⁷ was modified as described¹⁸: Briefly, isolated nuclei in 0.5 mM CaCl₂, 0.5 mM MgCl₂, 25 mM KCl, 0.14 mM spermidine, 1 mM Tris-HCl, pH 7.5 (buffer I) were sheared in the French pressure cell at 10,000 psi. The suspension was dialysed for 4 hrs against 4 l of 10 mM Tris-HCl, pH 7.5 and then loaded onto a column of ECTHAM-cellulose (1.6 x 8.0 cm). Chromatin was eluted as two clearly distinct peaks by a combined salt-pH gradient prepared from 40 ml 10 mM Tris-HCl, pH 7.5, and 40 ml 450 mM NaCl, 50 mM Tris-HCl, pH 8.8. To fractionate chromatin by differential centrifugation, nuclei sheared in buffer I were centrifuged for 7 min at

1,000 g. The supernatant contained the template active fraction contaminated with ribonucleoprotein particles, while the template inactive fraction was localized in the pellet.

Rat liver DNA was isolated from purified nuclei as described¹⁸.

Transcription studies. Transcription of chromatin by an excess of *E. coli* RNA polymerase was performed as described¹³ except that $(\text{NH}_4)_2\text{SO}_4$ was replaced by 37.5 μmole NaCl. The amount of RNA polymerase chosen (8 μg of enzyme per 5 μg chromatin DNA) was based on prior experiments designed to determine the saturation level of the high affinity binding sites on chromatin^{13,18}. The compounds to be tested were present either during the whole reaction period or were added simultaneously with the triphosphates and rifampicin.

Transcription of chromatin (5 μg) with 6 μg calf thymus RNA polymerase II was performed in a final volume of 250 μl containing 12.5 μmole Tris-HCl, pH 7.9, 0.50 μmole MnCl_2 , 25 μmole NaCl, 0.05 μmole MgCl_2 , 5.8 μmole $(\text{NH}_4)_2\text{SO}_4$, 0.5 μmole 2-mercaptoethanol, 25 nmole of each ATP, CTP, and GTP, and 5 μCi [^3H] UTP. The incubation was carried out for 15 min at 37° and stopped with 5% TCA. To assay specifically the binding of the calf thymus enzyme to the template, chromatin was preincubated in a volume of 200 μl containing all above listed reagents except the triphosphates. These were added after 15 min simultaneously with 75 μmole $(\text{NH}_4)_2\text{SO}_4$ in 50 μl and the incubation was continued for 15 min.

RESULTS

To analyze the effect of polar organic compounds on *in vitro* transcription on chromatin, I have made use of the observation of So and Downey¹⁹ that although *E. coli* RNA polymerase is highly sensitive to inhibition by rifampicin, on binding to DNA and formation of a phosphodiester bond, the enzyme becomes resistant to the antibiotic. The general approach followed has been to form an RNA polymerase-chromatin-complex and then to incubate further in the

presence of the four ribonucleoside triphosphates added simultaneously with rifampicin. Under these conditions, only those complexes able to initiate rapidly would be protected from rifampicin inhibition.

The effect of various polar organic compounds on the overall transcription of chromatin derived from Friend-virus-infected erythroleukemia cells with *E. coli* RNA polymerase is shown in Table I. Ten compounds were found to stimulate transcription although their effective concentrations on a molar basis appear to differ. All of these compounds (with the exception of 2-methyl-2,4-pentanediol which has not been tested) have been found to induce hemoglobin synthesis in Friend cells¹. The polar compounds have similar effects

Table I

Effect of polar organic compounds on the overall transcription of chromatin from Friend cells by *E. coli* RNA polymerase and RNA polymerase II from calf thymus. Final concentrations of the compounds are given in mM.

<u>Reagent added</u>	<u>Conc.</u>	<u>Enzyme</u>	<u>cpm</u>	<u>% of control</u>
none	-	<i>E. coli</i>	6,320	(100)
DMSO	640	<i>E. coli</i>	9,430	149
2-Methyl-2,4-pentanediol	420	<i>E. coli</i>	9,160	145
Triethylene glycol	330	<i>E. coli</i>	10,520	166
N-Methylformamide	330	<i>E. coli</i>	8,650	137
N-Methylacetamide	270	<i>E. coli</i>	8,760	139
Pyrrolidone-(2)	230	<i>E. coli</i>	8,130	129
N,N-Dimethylacetamide	230	<i>E. coli</i>	8,570	136
1-Methyl-pyrrolidone-(2)	200	<i>E. coli</i>	8,370	132
Acetamide	160	<i>E. coli</i>	9,040	134
1-Methyl-2-piperidone	40	<i>E. coli</i>	8,810	139
none	-	calf thymus	7,320	(100)
DMSO	640	calf thymus	11,030	151
2-Methyl-2,4-pentanediol	420	calf thymus	10,340	141
Triethylene glycol	330	calf thymus	12,270	168

on overall RNA synthesis when chromatin is transcribed with calf thymus RNA polymerase II. To investigate the increase in RNA synthesis in detail DMSO was chosen as a representative. Addition of DMSO to the reaction mixture up to a concentration of 0.6 to 0.9 M increases total RNA synthesis (Fig.1). At DMSO concentrations greater than 0.9 M transcription is progressively inhibited. Transcription by *E. coli* RNA polymerase and by calf thymus RNA polymerase II appear to be equally influenced (Table I and Fig. 1). In the experiments shown in Table I and Fig. 1, the time allowed for RNA synthesis was 15 min. When much shorter incubation periods (50 sec) are chosen, similar results are obtained indicating that transcription does not cease by the end of the incubation period.

To determine the template specificity of the effect of DMSO on transcription, I tested chromatin derived from various tissues. The results (Table II) show that DMSO increases RNA synthesis also on these templates, although the stimulatory effect varies in the range from 25 to 50%.

Fig. 1: Transcription on chromatin from Friend cells with *E. coli* RNA polymerase (○—○) and calf thymus RNA polymerase II (●—●) as a function of the DMSO concentration. The amount of RNA synthesis in the absence of DMSO was taken as 100% (7,280 cpm for transcription by the *E. coli* and 8,340 cpm for transcription by the mammalian enzyme).

Table II

Effect of DMSO on the overall transcription of chromatin from various sources with E. coli RNA polymerase. The DMSO concentration was 0.6 M.

<u>Chromatin source</u>	<u>cpm - DMSO</u>	<u>cpm + DMSO</u>	<u>% of control</u>
Friend cells	7,620	10,090	132
rat liver	8,650	11,720	135
hen oviduct	6,960	9,890	142
rat spleen	6,070	8,420	139
hen brain	5,860	7,510	128

Table III

Effect of DMSO on the overall transcription of separated template active and template inactive fractions from rat liver chromatin. The DMSO concentration was 0.6 M.

<u>Fractionation method</u>	<u>Chromatin fraction</u>	<u>cpm - DMSO</u>	<u>cpm + DMSO</u>	<u>% of control</u>
ECTHAM-cellulose	templ. active	9,650	11,590	120
ECTHAM-cellulose	templ. inactive	3,210	4,840	151
differential centrifug.	templ. active	8,870	10,530	119
differential centrifug.	templ. inactive	2,430	3,790	156

Interphase chromatin consists of two forms: a diffuse, transcriptionally active form and a condensed, transcriptionally inactive one^{5,20}. In order to investigate whether DMSO effects RNA synthesis on these two templates differentially, chromatin from rat liver and hen oviduct were fractionated into template active and template inactive fractions by ECTHAM-cellulose chromatography and differential centrifugation. The results for rat liver chromatin (Table III) show that the effect of DMSO on transcription depends strongly on the template structure. RNA synthesis on the template active fraction obtained by either method is increased only slightly (by a factor of approximately

1.2), while transcription on the template less active portion is stimulated by a factor of approximately 1.5. Identical results were obtained with the respective fractions from hen oviduct.

To test whether DMSO increases the initiation or elongation of RNA chains, *E. coli* RNA polymerase was preincubated with chromatin in the presence or absence of DMSO. Table IVA shows that addition of DMSO simultaneously with the triphosphates and rifampicin increases RNA synthesis by about 45%. The addition of DMSO immediately after rifampicin increases transcription by 43%, indicating that with the simultaneous addition of rifampicin and DMSO, the DMSO is unlikely to act before rifampicin inhibits the polymerase. When DMSO is also present during the preincubation period, RNA

Table IV

Effect of DMSO on preincubation of *E. coli* RNA polymerase with rat liver chromatin (A) and rat liver DNA (B), and calf thymus RNA polymerase II (C) with rat liver chromatin. When stated, DMSO was present at a concentration of 0.6 M in the final reaction volume of 250 μ l.

<u>Enzyme</u>	<u>Preincubation</u>	<u>Additions</u>	<u>cpm</u>
A. <i>E. coli</i>	E + chromatin	rif + NTPs	7,540
<i>E. coli</i>	E + chromatin	rif + NTPs + DMSO	10,920
<i>E. coli</i>	E + chromatin + DMSO	rif + NTPs	11,160
B. <i>E. coli</i>	E + DNA	rif + NTPs	40,200
<i>E. coli</i>	E + DNA	rif + NTPs + DMSO	43,000
<i>E. coli</i>	E + DNA + DMSO	rif + NTPs	71,800
C. calf thymus	E + chromatin	$(\text{NH}_4)_2\text{SO}_4$ + NTPs	4,180
calf thymus	E + chromatin	$(\text{NH}_4)_2\text{SO}_4$ + NTPs + DMSO	6,370
calf thymus	E + chromatin + DMSO	$(\text{NH}_4)_2\text{SO}_4$ + NTPs	6,710

synthesis increases by about 48%. Thus, the major effect of DMSO is to stimulate transcription from performed rifampicin-resistant complexes.

Table IVB shows that with deproteinized rat liver DNA as template the addition of DMSO simultaneously with rifampicin increases RNA synthesis by only 7.5%. In contrast addition of the compound prior to rifampicin increases transcription markedly. These results confirm previous experiments^{21,22}, which indicate that when DNA was used as template DMSO stimulates primarily the formation of the rifampicin-resistant initiation complex.

To analyse the influence of DMSO on the formation of the initiation complex from calf thymus RNA polymerase II and chromatin, I made use of its sensitivity to dissociation by solutions of high ionic strength. Initiation of the mammalian polymerase is completely inhibited when $(\text{NH}_4)_2\text{SO}_4$ at a concentration of 0.3 M was present prior to the addition of the substrates. However, when $(\text{NH}_4)_2\text{SO}_4$ is added to the preincubated enzyme at 0.3 M simultaneously with the triphosphates, incorporation is reduced only by 31%. Table IV C shows that DMSO added simultaneously with the triphosphates and $(\text{NH}_4)_2\text{SO}_4$ increases RNA synthesis by about 30%. The same increase in incorporation occurs when DMSO is present during the whole reaction period. This indicates that DMSO has little influence on the formation of the salt insensitive initiation complex from chromatin and RNA polymerase II.

DISCUSSION

The template properties of chromatin are used in the present study to measure the effect of polar organic compounds on chromatin structure. Investigations from other laboratories¹⁻⁴ have indicated that DMSO which may be considered as a representative of these compounds induces erythroid differentiation by an alteration in the program of transcription. The present study shows that these compounds stimulate transcription on isolated chromatin by an excess of added

RNA polymerase. RNA synthesis is increased (i) by polar organic compounds, which are rather dissimilar with respect to their chemical structure, (ii) using a bacterial and a eukaryotic RNA polymerase and (iii) on chromatin templates from a variety of sources. These results indicate that the target of these compounds is the chromatin template. The sensitivity of initiation by E. coli RNA polymerase to rifampicin and by the calf thymus enzyme to solutions of high ionic strength is used to study the effect of DMSO on the initiation of RNA chains. DMSO has very little influence on the binding of either RNA polymerase to form a stable complex and the first phosphodiester bond, but increases RNA synthesis mainly by releasing transcription from restrictions during elongation. In this regard, it is of interest that recent studies^{13,18} have shown that formation of the initiation complex with E. coli RNA polymerase is facilitated in chromatin as compared to deproteinized DNA. After separation of isolated chromatin into a template active and template inactive fraction, RNA synthesis on the transcriptionally more restricted fraction is increased to a greater extent than on the template active portion, indicating a direct relationship between the degree of template restriction and the stimulation of RNA synthesis by DMSO.

The addition of organic compounds much less polar than water alter the physicochemical properties of the medium. For these reasons, it is readily apparent that the understanding how these compounds effect chromatin structure requires a more detailed knowledge of the type of forces maintaining chromatin organization. A recently formulated model envisages chromatin as organized discontinuously in subunits with the DNA wound on the outside of a repeat structure of assembled histones^{6-9,23}. It is suggested that the basic histone segments on the outside are the primary site of interaction with the DNA, while the hydrophobic bonds in the apolar segments function in histone assembly and subunit conformation⁹⁻¹¹. The addition of organic compounds much less polar than water is expected to reduce the dielectric constant of the solvent, which will weaken the hydrophobic inter-

actions. This weakening of the hydrophobic bonds is suggested to cause a release of RNA synthesis on chromatin from the constraints imposed by histones. Physicochemical studies by Lapeyre and Bekhor¹² have shown a more B-type secondary DNA conformation and a decreased thermal stability of chromatin in the presence of DMSO. These results support the suggestion that the increase in RNA synthesis by polar organic compounds is mediated by a relaxation of chromatin structure. As the interactions between DNA and the basic histone segments involve mainly salt linkages, which should be strengthened by lowering the dielectric constant of the medium, the observation of an increased RNA synthesis by polar organic compounds would seem to show that the effect of these compounds on hydrophobic bonds is more important than on salt linkages.

It is tempting to suggest that also the induction of erythroid differentiation of Friend cells by polar organic compounds is mediated by a relaxation of chromatin structure, which causes a change in the program of transcription. This suggestion receives support from the requirement of adequate intracellular DMSO concentrations for the induction of Friend cell differentiation³, from the absence of globin gene transcripts in noninduced cells⁴, and from the observation that a great number of genes have to be expressed during erythroid differentiation.

ACKNOWLEDGEMENTS

The author thanks I. Seidel for competent technical assistance. This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

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