Partial characterization of the mode of inhibition of *Escherichia coli* RNA polymerase by the mixed disulfide, CoASSG[1]

WILLIAM C. H. BEES AND PETER C. LOEWEN

Department of Microbiology, University of Manitoba, Winnipeg, Man., Canada R3T 2N2

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The coenzyme A – glutathione mixed disulfide (CoASSG), when complexed with iron, is capable of inhibiting the RNA polymerase of *Escherichia coli*. A modified procedure involving a short time of exposure to high salt allowed the reliable preparation of CoASSG–Fe which was active in inhibiting RNA polymerase. The CoASSG–Fe complex acted as a noncompetitive inhibitor for the incorporation of all four nucleoside triphosphates but had a greater effect on GMP and CMP incorporation than AMP and UMP incorporation. Neither temperature nor ionic-strength changes affected CoASSG–Fe inhibition, and the use of rifampicin showed that CoASSG–Fe did not inhibit either the initiation or elongation processes of the polymerase. CoASSG–Fe was a more effective inhibitor at low DNA-template concentrations and it was more effective in inhibiting the incorporation of CMP and GMP on simple dG-dC containing templates and the asymmetric polymer poly d(T-C)·poly d(G-A). The inhibition of transcription of poly d(I-C) was less effective than the inhibition of transcription of poly d(G-C). Equilibrium dialysis in microdialysis cells showed that CoASSG–Fe could associate with DNA in the absence of RNA polymerase.

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Quand il forme un complexe avec le fer, le disulfure mixte Coenzyme A – glutathion (CoASSG) est capable d'inhiber la RNA polymérase d'*Eschericia coli*. Une technique modifiée, impliquant une courte exposition à une concentration saline élevée, permet d'obtenir une préparation CoASSG–Fe capable d'inhiber la RNA polymérase. Le complexe CoASSG–Fe inhibe de façon non compétitive l'incorporation des quatres nucléosides triphosphatés, mais il exerce un effet plus grand sur l'incorporation du GMP et du CMP que sur l'incorporation de l'AMP et de l'UMP. Ni les changements de température ni les changements de la force ionique n'affectent l'inhibition exercée par le CoASSG–Fe et l'emploi de la rifampicine montre que le CoASSG–Fe n'inhibe pas les processus d'initiation ou d'élongation de la polymérase. Le CoASSG–Fe est un inhibiteur plus efficace en présence de faibles concentrations de DNA matriciel et il est encore plus efficace pour inhiber l'incorporation du CMP et du GMP sur des moules simples contenant dG-dC et sur le polymère asymétrique poly d(T-C)·poly d(G-A). L'inhibition de la transcription du poly d(I-C) est moins importante que l'inhibition de la transcription du poly d(G-C). La dialyse à l'équilibre dans des cellules à microdialyse montre que le CoASSG–Fe pourrait s'associer avec le DNA en absence de RNA polymérase.

[Traduit par le journal]

Introduction

CoASSG has been characterized in a number of organisms (1–3), including most recently *Escherichia coli* (4), but no conclusion has yet been drawn concerning its *in vivo* role. In rat liver (5) the CoASSG levels were very low, arguing against it being a storage form of CoA, while in yeast (1) and *E. coli* (4) the mixed disulfide was a major component of the CoA pool and even varied with certain metabolic changes (6, 7) suggesting that it may be a CoA storage form during certain stages of growth. In addition, a preliminary report (8) has implicated CoASSG in the regulation of RNA polymerase from *E. coli*.

That CoASSG may act to inhibit transcription was originally suggested by the accumulation of the mixed disulfide during growth into stationary phase and after a shift to anaerobic growth (6) (situations where transcription was known to terminate). Subsequent work showed that glucose starvation, as well as a number of drugs and metabolic poisons, elicited a similar increase in CoASSG (7). Since ppGpp pools also increased in response to some of these disruptions, and

ABBREVIATIONS: CoASSG, coenzyme A — glutathione mixed disulfide; TEAB, triethylammonium bicarbonate; $A_{250:260}$, absorbance ratio at 250 and 260 nm.

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it was known that ppGpp could inhibit *E. coli* RNA polymerase, we undertook to expand upon the preliminary report (8) and more fully characterize the mechanism by which CoASSG could inhibit transcription.

When CoASSG was freshly isolated from the cell paste of *E. coli*, it was found to be complexed with iron (CoASSG–Fe), to exhibit an elevated $A_{250:260}$ and to inhibit RNA polymerase (4, 8). Upon storage, the inhibitory activity decreased while at the same time the $A_{250:260}$ ratio dropped. While the inhibition of RNA polymerase by CoASSG required the presence of iron (4) and it seemed that old CoASSG–Fe solutions, and even authentic CoASSG, could be reactivated by the addition of iron, this has proven to be an unreliable procedure for isolating a CoASSG–Fe complex which is active in inhibiting RNA polymerase. This work, in part, reports a modified procedure for isolating active CoASSG–Fe.

The preliminary report showed CoASSG–Fe to act as a noncompetitive inhibitor for ATP incorporation, possibly interacting with the RNA polymerase–DNA complex (8). A more detailed study of the mechanism of RNA polymerase inhibition has been carried out in the present work which indicates that CoASSG forms an unstable complex with iron, possibly involving a strained conformation, which can interact directly with the DNA template, even in the absence of RNA polymerase, to inhibit transcription.

Materials and Methods

Chemicals and Enzymes

All common biochemicals, Sephadex G-50 and DEAE Sephadex A-25, were obtained from Sigma as was *E. coli* RNA polymerase (EC 2.7.7.6). All radioactive isotopes were obtained from New England Nuclear. The synthetic polymers poly d(T-C) · poly d(G-A) and poly d(T-G) · poly d(C-A) were a gift from Dr. H. G. Khorana.

Purification of CoASSG–Fe

The procedure for isolating CoASSG–Fe was as previously described (4, 6) but with three modifications which reduced the time necessary for the purification and which reduced the exposure of the extract to high salt. As described, ten 1-L cultures of *E. coli* B were grown into stationary phase, collected by centrifugation, and extracted with 1 *M* formic acid. After neutralization, the extract was diluted fivefold with water (to 1 L) and charged on a 2 × 30 cm column of DEAE Sephadex A-25 equilibrated with deionized water. After charging (which required 6–8 h), the column was washed overnight with 250 mL of 0.2 *M* TEAB pH 7.6. A linear gradient of 500 mL 0.2 *M* TEAB pH 7.6 and 500 mL 1.5 *M* TEAB was applied (6 h) and the peak corresponding to CoASSG was pooled and concentrated to dryness by evaporation under vacuum (2 h). The residue was dissolved in 1 mL of 50 m*M* Tris–HCl pH 7.6 with 2.5 m*M* $Fe_2(SO_4)_3$ and purified further on a 1 × 100 cm column of Sephadex G-50 equilibrated with 100 m*M* TEAB pH 7.6 (overnight). The peak of CoASSG–Fe was collected, concentrated by evaporation under vacuum, and stored in distilled water at −76°C. The ratio of absorbance at 250 and 260 nm was usually above 0.95.

RNA Polymerase Assay

The standard RNA polymerase assay reaction mixture (0.06 mL) contained 50 m*M* Tris–HCl pH 7.6, 10 m*M* $MgCl_2$, 40 m*M* KCl, 0.2 m*M* each of the sodium salts of ATP, GTP, CTP, and usually [^{3}H]UTP (0.182 mCi/mmol (1 Ci = 37 GBq)), 1.1 μg RNA polymerase (0.67 units/μg), and 92 μg/mL salmon sperm DNA. The assay was usually started following any preincubation stage by the addition of the nucleoside triphosphate mixture. Aliquots of 10 μL were spotted on 1.5 × 2 cm pieces of Whatman No. 1 filter paper which were then washed at 4°C in 10% trichloroacetic acid – 1% sodium pyrophosphate for 10 min, two times in 5% trichloroacetic acid – 0.5% sodium pyrophosphate for 5 min, followed by 95% ethanol and drying. Any changes to this procedure are noted in the individual experiments.

Equilibrium Dialysis

Migration of [^{32}P]CoASSG–Fe across a membrane to associate with DNA was followed using a slight modification of the microdialysis cells described by Englund *et al.* (9). The membrane was standard dialysis tubing pretreated by boiling for 30 min in 5% sodium carbonate – 50 m*M* ethylenediaminetetraacetic acid and for another 30 min in distilled water. The volume of each chamber was 100 μL and aliquots of 10 μL were removed from both chambers at various times, dried on glass-fiber filters, and the radioactivity determined by liquid scintillation. The buffer used in all experiments was 25 m*M* Tris–HCl pH 7.6.

Results

Isolation of CoASSG–Fe which Inhibits RNA Polymerase

The preliminary report (8) describing the inhibition of RNA polymerase by CoASSG–Fe also noted that the inhibitory activity was unstable and that the loss of this activity could be correlated with a drop in the $A_{250:260}$ ratio. It was subsequently shown (4) that CoASSG–Fe was a mixed disulfide, CoASSG, in a cómplex with iron which was responsible for the elevated $A_{250:260}$ ratio. As shown in Table 1, the presence of iron was also necessary for CoASSG to inhibit RNA polymerase. A batch of CoASSG–Fe which had lost a significant portion of its inhibitory activity during storage had its inhibitory activity more than doubled by incubation with ferric sulfate prior to the addition of RNA polymerase to the assay mixture. Unfortunately, this reactivation process was not successful for every batch of CoASSG–Fe and the gel-filtration procedure described (4) for isolating the CoASSG–Fe complex also was not successful for all preparations. Among the various batches which could not be reactivated, the only similarity was that they had been stored at −20°C or −76°C for more than 1 month while those preparations which could be activated were freshly isolated preparations.

The procedure followed for the isolation of CoASSG–Fe was therefore modified with the objective of increasing the reliability with which active preparations were produced. The primary goal was to speed up the procedure and as described in Materials and Methods,

TABLE 1. Effect of the preincubation of CoASSG–Fe with iron on the inhibition of RNA polymerase by CoASSG–Fe

Expt. No.	Addition*	[^{3}H]UMP incorporated (pmol/10 min)	% inhibition
1	H_2O	36.3	—
2	$Fe_2(SO_4)_3$ (100 μM)	33.6	7.4
3	$FeSO_4$ (100 μM)	15.2	58.1
4	CoASSG–Fe (75 μM)	31.1	14.3
5	$Fe_2(SO_4)_3$ (100 μM) + CoASSG–Fe (75 μM)	24.8	31.6

*Assays were as described in Materials and Methods except that additions were preincubated with DNA and CoASSG–Fe for 5 min, then RNA polymerase for 5 min (both at 37°C) after which the reaction was started by the addition of the nucleoside triphosphates.

TABLE 2. Effect of CoASSG–Fe on the apparent K_m of RNA polymerase for the nucleoside triphosphates (from Fig. 1)

Expt. No.	Nucleotide	Apparent K_m,* μM	Literature K_m values, μM	
			Ref. 10†	Ref. 11‡
1	ATP	14.0	33.8	16
2	ATP + CoASSG–Fe (12 μM)	22.9		
3	ATP + CoASSG–Fe (60 μM)	23.0		
4	GTP	10.5	32.3	27
5	GTP + CoASSG–Fe (12 μM)	53.8		
6	CTP	8.5	17.8	15
7	CTP + CoASSG–Fe (12 μM)	50.0		
8	UTP	8.8	33.8	13
9	UTP + CoASSG–Fe (12 μM)	8.7		

*Salmon sperm DNA.
†T4 bacteriophage DNA.
‡*Micrococcus lysodeikticus* DNA.

the modified procedure took just 48 h once the cells were grown. The three significant changes made in the actual protocol were the use of DEAE Sephadex A-25 equilibrated with water rather than TEAB in the first step, the addition of iron to CoASSG prior to the final Sephadex G-50 column, and the replacement of the second DEAE Sephadex A-25 column with a Sephadex G-50 column. Using this modified procedure, preparations of CoASSG–Fe with $A_{250:260}$ ratios greater than 0.95 and with inhibitory activity could be reliably isolated.

Nucleoside Triphosphate Competition with CoASSG–Fe

CoASSG–Fe was shown to be a noncompetitive inhibitor for ATP incorporation by RNA polymerase (8). As shown in Fig. 1, the same conclusion can be drawn for the other nucleoside triphosphates. It should be noted that the interactions of GTP and CTP with RNA polymerase were affected to a greater extent than the interactions of ATP and UTP. This discrepancy between the K_m values of RNA polymerase for the various nucleoside triphosphates observed in this work and those reported by other workers (10, 11, and Table 2) is probably a result of the different templates which were used.

Effect of Ionic Strength and Temperature on the Inhibition of RNA Polymerase by CoASSG–Fe

It has been shown that RNA polymerase does not reinitiate RNA synthesis as well at KCl concentrations between 0.05 and 0.1 M as it does at 0.2 M KCl (12), suggesting a conformational change in the enzyme which affects protein – nucleic acid interactions (13). As shown in Fig. 2*a*, the level of inhibition of transcription by CoASSG–Fe was not affected by increasing concentrations of KCl. The ratio of incorporation without and with CoASSG–Fe remained constant over a range of KCl concentrations from 30 to 430 mM.

Similarly, the inhibition of RNA polymerase by CoASSG–Fe was not changed over a temperature range from 25 to 38°C (Fig. 2*b*). Above 38°C, however, there was a marked decrease in the amount of inhibition by CoASSG–Fe. Whether this decrease in inhibition was due to faster inactivation of CoASSG–Fe at the higher temperatures or to a decreased interaction of CoASSG–Fe with the transcription complex (or DNA) was not determined.

Inhibition of RNA Polymerase by CoASSG–Fe in the Presence of Rifampicin

When tested with rifampicin in the reaction mixture (14), CoASSG–Fe was shown to affect neither the

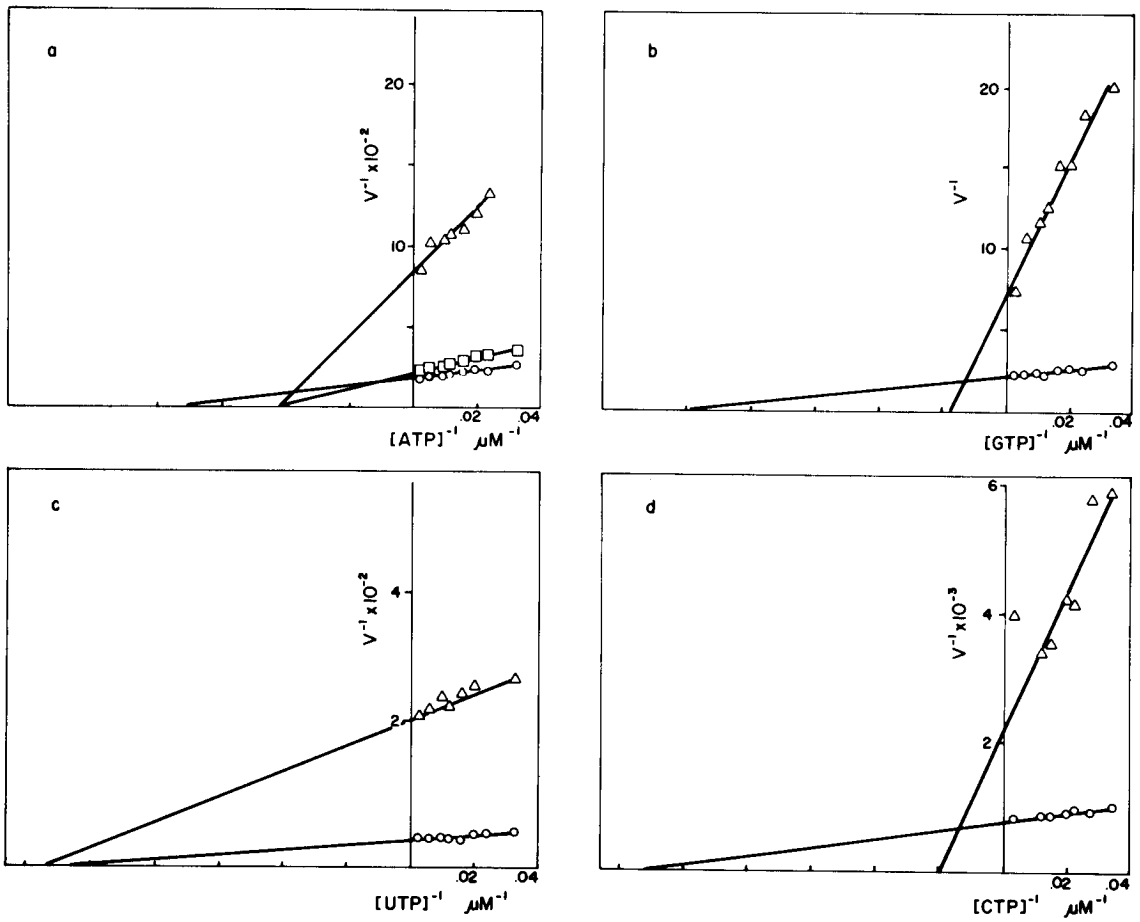

FIG. 1. Effect of nucleoside triphosphate concentration on the inhibition of RNA polymerase by CoASSG–Fe presented in Lineweaver–Burke form. Assay conditions were as described in Materials and Methods except that 20 μg/mL salmon sperm DNA and 200 m*M* KCl were used. The concentration of the nucleoside triphosphate being studied was varied from 0.03 to 0.4 m*M* and the remaining nucleoside triphosphates were maintained at 0.1 m*M*. [^{3}H]UTP was used in parts *a*, *b*, and *d* while [^{3}H]ATP was used in part *c*. DNA and RNA polymerase were incubated with CoASSG–Fe or water for 5 min prior to the addition of the nucleoside triphosphate mixture. All plots represent an average of three assays. (*a*) ATP varied: □, CoASSG–Fe (12 μM); △, CoASSG–Fe (60 μM); and ○, water. (*b*) GTP varied: △, CoASSG–Fe (12 μM); and ○, water. (*c*) UTP varied: △, CoASSG–Fe (12 μM); and ○, water. (*d*) CTP varied: △, CoASSG–Fe (12 μM); and ○, water.

initiation nor the elongation stages of transcription. With rifampicin added to prevent any reinitiation, the rate of elongation of RNA chains by RNA polymerase was found to be fractionally faster in the presence than in the absence of CoASSG–Fe (Fig. 3). As well, CoASSG–Fe did not significantly affect the formation of rifampicin-resistant RNA polymerase – DNA complexes (Table 3).

When combined with the insensitivity of the inhibitory activity to changes in KCl concentration and to changes in temperature below 38°C, this data suggested that CoASSG–Fe did not directly interact with the RNA polymerase. Instead, an interaction with some other component of the reaction mixture, possibly the DNA template, was suggested by the noncompetitive nature of the inhibition of incorporation.

Effect of DNA Concentration and Sequence on CoASSG–Fe Inhibition of RNA Polymerase

As shown in Fig. 4, CoASSG–Fe more effectively inhibited RNA polymerase at low DNA concentrations with the extent of inhibition increasing as the log of the CoASSG : base pair ratio. At small CoASSG : base pair ratios, CoASSG–Fe would appear to be interacting cooperatively (8) at preferred locations on the template resulting in a plateau of 40% inhibition.

The extent of inhibition of the transcription of templates with different base compositions is summarized in Table 4. The incorporation of CMP and GMP was inhibited more than the incorporation of UMP and AMP when simple polymers containing just dG and dC or dA and dT were used as templates (experiments 1–9 in Table 4). Two exceptions were the greater in-

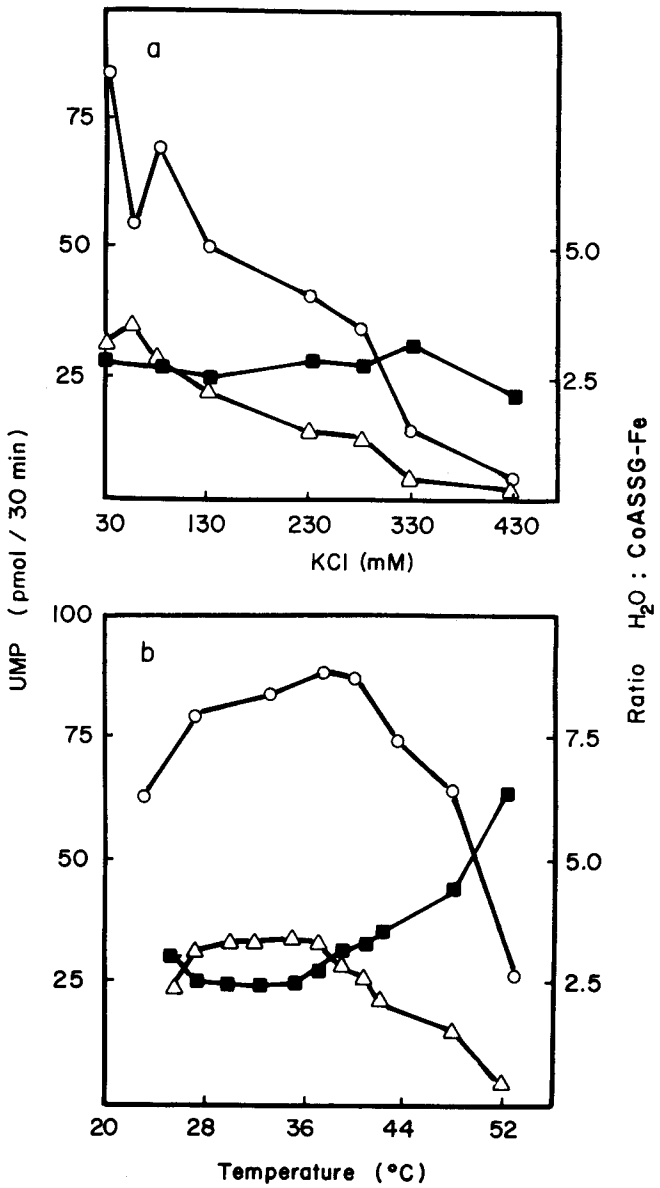

FIG. 2. Effect of (*a*) increasing ionic strength and (*b*) temperature on the inhibition of RNA polymerase by CoASSG–Fe. Assay conditions were as described in Materials and Methods. △, UMP incorporated in the presence of CoASSG–Fe (picamoles per 30 min); ○, UMP incorporated in the absence of CoASSG–Fe (picamoles per 30 min); and ■, ratio of incorporation in the absence and presence of CoASSG–Fe.

hibition of AMP incorporation on poly dT and the lower inhibition of GMP incorporation on poly d(I-C). This same pattern was observed in the transcription of the asymmetric poly d(pyrimidine)·poly d(purine) template, poly d(T-C)·poly d(G-A), which has been shown to be transcribed asymmetrically by RNA polymerase (15). The transcription of the non-asymmetric polymers such as poly d(T-G)·poly d(A-C) and salmon sperm DNA was inhibited even less and the incorporation of all four nucleotides was affected equally. Increasing inhibition of transcription by CoASSG–Fe was observed in the series of natural templates, salmon sperm DNA, T4 bacteriophage DNA, *E. coli* DNA and T7 bacteriophage DNA but this trend could not be correlated to changes in the base composition of the DNAs. Possibly asymmetric base distributions localized on the different templates are responsible for the trend.

Effect of Spermidine on CoASSG–Fe Inhibition of Transcription

Spermidine has been shown to interact with DNA

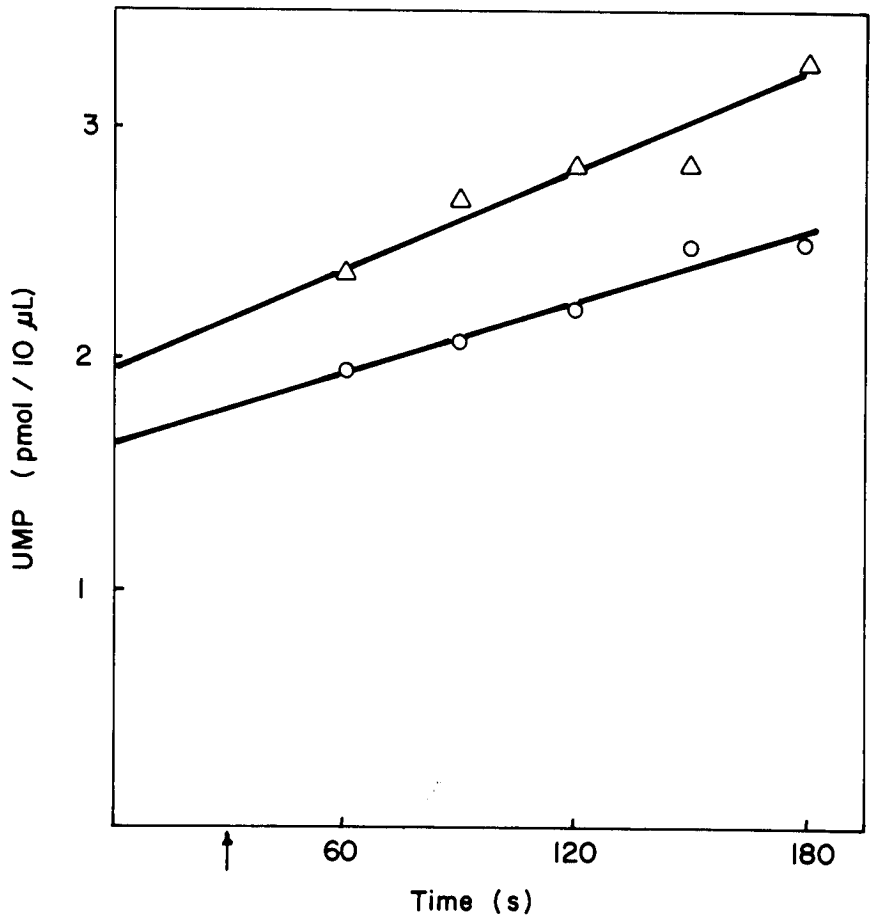

FIG. 3. Effect of CoASSG–Fe on RNA chain elongation by *E. coli* RNA polymerase. Two standard assay mixtures were prepared and incubated at 32°C. After 30 s, rifampicin (200 μg/mL) was added to one mixture (○) and rifampicin (200 μg/mL) plus CoASSG-Fe (100 μ*M*) were added to the other mixture (△). Aliquots of 10 μL were removed at the indicated times.

in the minor groove of dA-dT rich regions of the DNA blocking RNA polymerase (16). As shown in Table 5, the effects of CoASSG–Fe and spermidine on transcription were nearly cumulative with the combined inhibition being 65% as compared to the theoretical cumulative effect of 73%. If spermidine has interacted just with the dA-dT rich regions, this would leave only the dG-dC rich regions for CoASSG–Fe interaction and the complex appears to be effective under these conditions.

TABLE 3. Effect of CoASSG–Fe on the formation of the DNA – RNA polymerase preinitiation complex

Expt. No.	Time of addition,* min: Rifampicin (150 μg/mL)	Time of addition,* min: CoASSG–Fe (100 μ*M*)	[^{3}H]UMP incorporated (pmol/10 min)
1	0	—	7.25
2	0	−5	6.87
3	0	0	5.59
4	−5	—	2.82

*DNA and RNA polymerase were incubated at 32°C for 5 min prior to the addition of the four nucleoside triphosphates to start the reaction at time 0. Rifampicin and CoASSG–Fe were added either prior to or at the time of starting the reaction as indicated.

Association of CoASSG–Fe with DNA

Nitrocellulose filters were shown to retain [^{32}P]CoASSG–Fe only after it had been incubated with a RNA polymerase – DNA mixture (8). RNA polymerase was required for the retention of CoASSG–Fe, and presumably DNA, on the filter and it was therefore not possible to directly study a CoASSG–Fe–DNA association using nitrocellulose filters. Instead, equilibrium dialysis in microdialysis chambers (9) was studied as a means of characterizing the apparent CoASSG–Fe–DNA interaction. The diffusion of [^{32}P]CoASSG–Fe from 'outside' to 'inside' or to the DNA-containing side of the membrane reached equilibrium in 18 h at 5°C with 80% of the label accumulating on the DNA side (Table 6). Dithiothreitol, which would reduce the disulphide linkage of CoASSG, eliminated the ac-

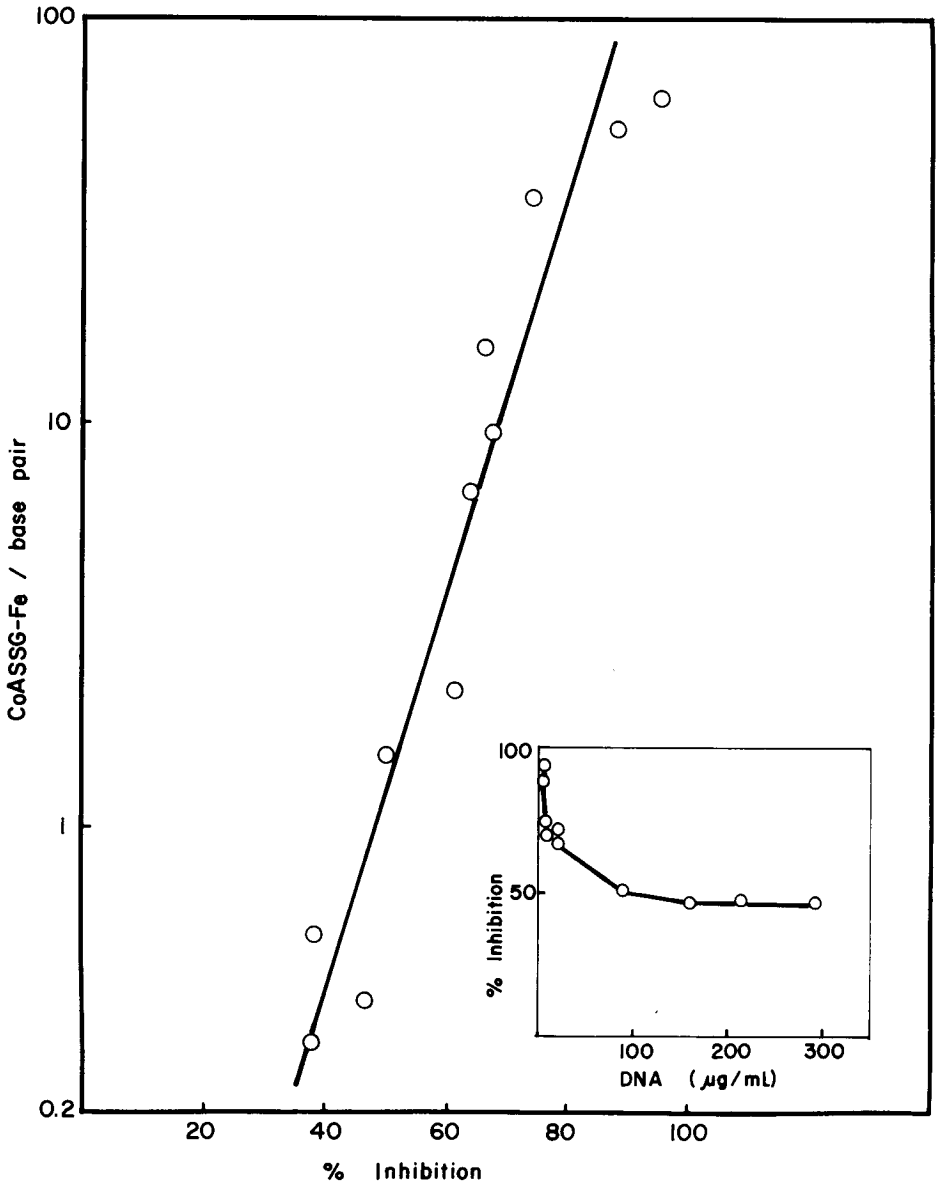

FIG. 4. Effect of DNA concentration on the inhibition of RNA polymerase by CoASSG–Fe. Assay conditions were as described in Materials and Methods except that DNA concentrations from 1.3 to 296 μg/mL of salmon sperm DNA were used with CoASSG–Fe at 116 μ*M*.

cumulation of excess CoASSG–Fe on the DNA side as did RNA polymerase which may compete with CoASSG–Fe for the DNA. As a control, [γ-^{32}P]ATP also reached equilibrium in 18 h but with an even distribution of label on both sides of the membrane. When the dialysis was followed over a longer period of time, the excess of CoASSG–Fe on the DNA side of the membrane began to decrease after 25 h and the label was evenly distributed after 50 h. Presumably, the CoASSG–Fe was inactivated as a result of dissociation of the ferric ion from the CoASSG and interaction with the DNA was no longer possible. When the equilibration was studied at 25°C, the migration of [^{32}P]CoASSG–Fe to the DNA side of the membrane occurred more quickly while migration of [^{32}P]CoASSG–Fe away from the DNA occurred more slowly (Fig. 5).

It should be noted that although all of the batches of CoASSG–Fe used in these experiments were effective in inhibiting RNA polymerase, different amounts of association of CoASSG–Fe with DNA were observed among the different batches perhaps because the DNA association is more sensitive to changes in the CoASSG–

TABLE 4. Inhibition of the transcription of various templates by CoASSG–Fe

Expt. No.	Template	Nucleoside triphosphates	% inhibition
1	Poly d(G-C)	[^{3}H]CTP+GTP	49
2	Poly d(G-C)	[^{3}H]GTP+CTP	42
3	Poly dG·poly dC	[^{3}H]CTP+GTP	45
4	Poly dG·poly dC	[^{3}H]GTP+CTP	64
5	Poly d(I-C)	[^{3}H]GTP+CTP	25
6	Poly d(A-T)	[^{3}H]UTP+ATP	17
7	Poly d(A-T)	[^{3}H]ATP+UTP	20
8	Poly dA	[^{3}H]UTP	25
9	Poly dT	[^{3}H]ATP	53
10	Poly d(T-G)·poly d(C-A)	[^{3}H]GTP+UTP	28
11	Poly d(T-G)·poly d(C-A)	[^{3}H]UTP+GTP	25
12	Poly d(T-G)·poly d(C-A)	[^{3}H]CTP+ATP	24
13	Poly d(T-G)·poly d(C-A)	[^{3}H]ATP+CTP	27
14	Poly d(T-C)·poly d(G-A)	[^{3}H]GTP+ATP	50
15	Poly d(T-C)·poly d(G-A)	[^{3}H]ATP+GTP	34
16	Poly d(T-C)·poly d(G-A)	[^{3}H]CTP+UTP	45
17	Poly d(T-C)·poly d(G-A)	[^{3}H]UTP+CTP	26
18	Salmon sperm DNA (42% dG+C)	[^{3}H]UTP+CTP+GTP+ATP	23
19	T4 bacteriophage DNA (35% dG+dC-HOMe)	[^{3}H]UTP+CTP+GTP+ATP	52
20	*E. coli* DNA (50% dG+dC)	[^{3}H]UTP+CTP+GTP+ATP	60
21	T7 bacteriophage DNA (48% dG+dC)	[^{3}H]UTP+CTP+GTP+ATP	79

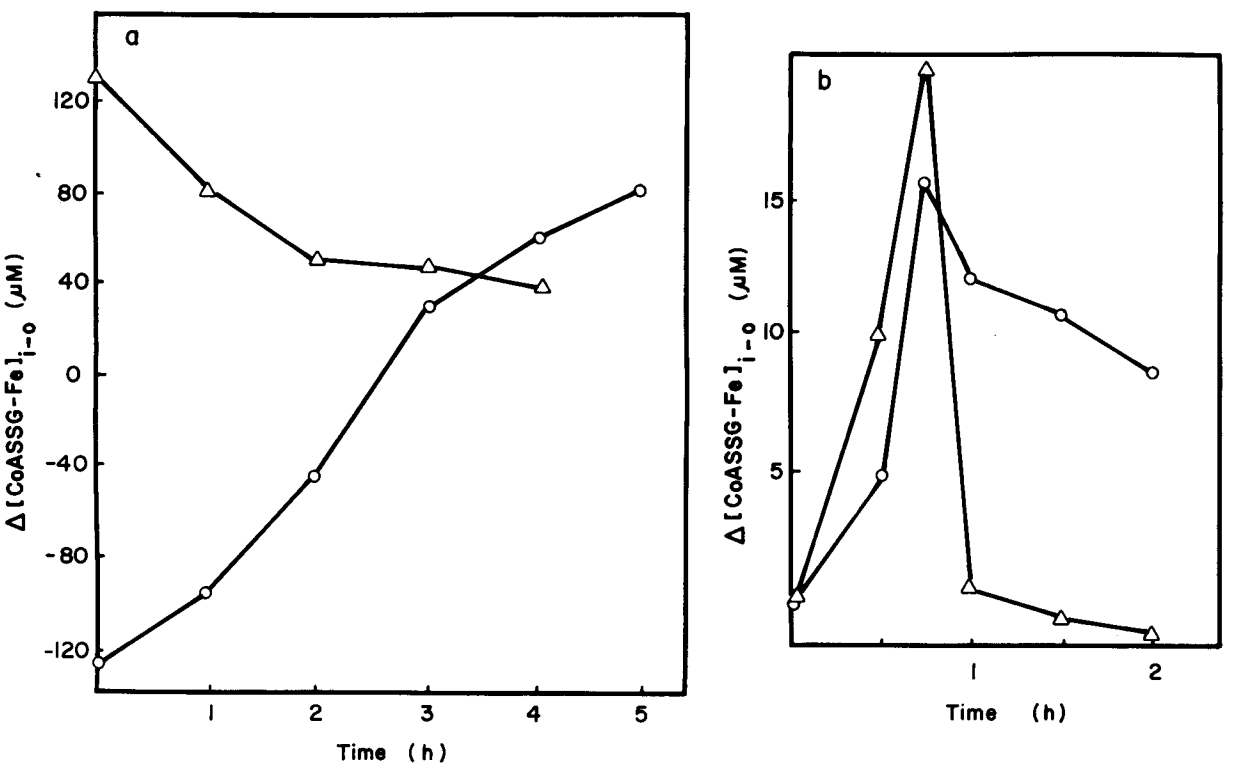

FIG. 5. (*a*) Migration of CoASSG–Fe during equilibrium dialysis at 25°C. [^{32}P]CoASSG–Fe (127 μM) was initially 'outside' (○) or 'inside' (△) the chamber containing the DNA (130 μg/mL) and 10-μL aliquots were removed at the indicated times. (*b*) Binding of CoASSG–Fe to poly d(G-C) (○) and poly d(A-T) (△) at 25°C. The CoASSG–Fe (95 μM) was initially at equal concentration both 'inside' and 'outside' of the chamber containing 40 μg/mL of either poly d(G-C) or poly d(A-T). Aliquots of 10 μL were removed at the times indicated.

TABLE 5. Effect of spermidine on the inhibition of RNA polymerase by CoASSG–Fe

Expt. No.	Addition*	% inhibition
1	CoASSG–Fe (100 μM)	45
2	Spermidine (16.6 mM)	51
3	CoASSG–Fe (100 μM) + spermidine (16.6 mM)	65

*CoASSG–Fe and spermidine were preincubated with the DNA for 5 min prior to the addition of RNA polymerase and nucleoside triphosphates to start the reaction.

TABLE 6. Association of CoASSG–Fe and ATP with DNA at equilibrium in microdialysis chambers at 5°C

Expt. No.	^{32}P-labelled nucleotide*	$[\text{Nucleotide}]_i - [\text{nucleotide}]_o$, μM
1	[^{32}P]CoASSG–Fe (106 μM) + RNA polymerase (96 units/mL)	38.8
2	[^{32}P]CoASSG–Fe (106 μM)	84.7
3	[^{32}P]CoASSG–Fe (106 μM) + dithiothreitol (1 mM)	8.2
4	[γ-^{32}P]ATP (50 μM)	−0.2

*Equilibrium was reached in 18 h. All of the nucleotide was 'outside' initially while the RNA polymerase in experiment 1 was mixed with the DNA 'inside.'

Fe structure. Associations ($[\text{CoASSG–Fe}]_i$–$[\text{CoASSG–Fe}]_o$) as large as 80 μM and as low as 14 μM were observed for different batches but in all cases the binding was reproducible and followed similar kinetics.

When the effect of increasing DNA concentration was studied in the microdialysis system, CoASSG–Fe appeared to migrate somewhat more quickly at DNA concentrations of 40 μg/mL, possibly the result of reduced ionic opposition. However, the association was also less stable and dissociation was more rapid at the low DNA concentration. Similarly, the use of poly d(A-T) (40 μg/mL) in place of salmon sperm DNA resulted in a rapidly formed but relatively unstable association, whereas poly d(G-C) (40 μg/mL) formed a more stable complex (Fig. 5*b*).

Attempts were also made to either isolate the CoASSG–Fe–DNA complex or show some degree of association using gel filtration and affinity chromatography, but with no success. When a mixture of CoASSG–Fe and salmon sperm DNA was separated on Sephadex G-50, no CoASSG–Fe was associated with the DNA peak. Similarly, no CoASSG–Fe was retained on DNA cellulose.

Discussion

The modified procedure described in this work was successful in isolating preparations of CoASSG–Fe which inhibited RNA polymerase on a reliable basis. It did not, however, eliminate the problem of the CoASSG–Fe complex being unstable and dissociating into an inactive form which usually could not be reactivated. An unknown factor appeared to be involved in the structure of CoASSG–Fe as it was isolated from the cell and it was this factor which was responsible for the inhibitory property of CoASSG–Fe. One possibility was the existence of a relatively unstable conformation adopted by CoASSG to allow the binding of iron which resulted in the formation of the active complex. In support of this interpretation was the change in the absorption spectrum of the adenine ring during the inactivation process (4) which indicated that the environment of this portion of the molecule was being changed. Alternatively, there may have been a labile component present which was necessary for the formation of the complex but which has not yet been identified.

A wide variety of compounds are known to inhibit RNA polymerase either by binding to the enzyme or to the DNA template with some acting as possible regulators *in vivo* (17). The preliminary report describing the inhibition of RNA polymerase by CoASSG–Fe did not address itself to the question of the mechanism of inhibition but the data could be interpreted to imply that it was the initiation stage of transcription which was being affected. The current work, however, shows that CoASSG–Fe does not interact directly with the enzyme to affect either the initiation or elongation stages of transcription. Rather, it affects the transcription process through a relatively weak interaction with the DNA template.

The more effective noncompetitive inhibition of GMP and CMP incorporation and the greater inhibition of transcription of dG-dC containing templates suggested a possible interaction of CoASSG–Fe with dG-dC rich regions of the DNA. The C-2 amino group of guanine was implicated in this interaction since the incorporation of GMP was inhibited less when poly d(I-C) was the template than when poly d(G-C) was

the template. The conformation of the DNA was also shown to be important in the CoASSG–Fe–DNA interaction by the inhibition of CMP and GMP incorporation being greater on an asymmetric poly d(purine)·poly d(pyrimidine) template than a similar template with a symmetric distribution of purines and pyrimidines on the two strands. The preference for dG-dC rich DNA and the possible minor groove interaction suggest two similarities to the interaction of actinomycin D with DNA (18) but extensive work remains to define the precise mechanism of interaction. Unfortunately, the instability of both active CoASSG–Fe and the CoASSG–Fe–DNA complex has seriously hampered studies aimed in this direction.

The metabolic significance of CoASSG–Fe inhibiting RNA polymerase activity remains unclear. Certain similarities were evident between the changes in CoASSG and the changes in ppGpp following metabolic disruption. It has been pointed out that changes in ppGpp levels do not occur fast enough to account for the rapid stop in transcription (19) and since the rate of change of CoASSG was similar to that of ppGpp, it is likely that some other factor is the primary cause of the very rapid termination of transcription after metabolic disruption. In spite of this, CoASSG–Fe, like ppGpp, can inhibit RNA polymerase *in vitro* and it may be acting as a secondary or tertiary means of transcription termination control which is not required under all conditions causing termination.

The presence of iron in the CoASSG–Fe complex further complicates a clear understanding of the physiological role of CoASSG. The environmental availability of iron, which may vary with other metabolic changes, may regulate the amount of CoASSG–Fe which is available to inhibit transcription. The possibility of CoASSG–Fe acting through a gene-specific interaction was not studied but the general attraction of the complex to dG-dC rich regions suggests a more general interaction.

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