JOURNAL OF BACTERIOLOGY, Oct. 1978, p. 96-103
0021-9193/78/0136-0096$02.00/0

Vol. 136, No. 1
Printed in U.S.A.

Role of the *dsdC* Activator in Regulation of D-Serine Deaminase Synthesis

MARIA C. HEINCZ AND ELIZABETH McFALL*
Department of Microbiology, New York University School of Medicine, New York, New York 10016

Received for publication 3 April 1978

The activator of the D-serine deaminase operon, the product of the *dsdC* gene, has been partially purified. It is reasonably stable to routine purification procedures in the presence of its ligand D-serine, but not in its absence. It loses activity upon dialysis in amino acid-free buffer, but activity is completely restored upon readdition of D-serine. It apparently functions purely as an activator; no repressor function could be demonstrated at suboptimal D-serine concentration. It is a transcriptional control element. The time required for in vitro transcription of D-serine deaminase mRNA, nearly 4 min, is similar to that for β-galactosidase. Since the β-galactosidase monomer is a much larger protein, this is surprisingly long.

There are at least three genetic loci specific to D-serine deaminase expression in *Escherichia coli* K-12: the enzyme's structural gene *dsdA* and its operator-promoter region *dsdO*, and a closely linked regulatory gene *dsdC*. The product of the regulatory gene is an activator, henceforth to be referred to as *dsdC* activator; it and the inducer D-serine are required for expression of the D-serine deaminase operon in wild-type cells. No enzyme is formed in vivo in $dsdO^+$ $dsdA^+$ cells which lack a functional *dsdC* gene (3), or in vitro in a cell-free system programmed with $dsdO^+$ $dsdA^+$ template DNA, unless an intact *dsdC* gene is also present. When synthesis of *dsdC* activator is programmed in vitro by $dsdC^+$ DNA, however, the $dsdO^+$ $dsdA^+$ template is expressed (8). We have assumed that the *dsdC* activator is a protein because of its ability to interact with D-serine and with its macromolecular site of action, and because of subunit mixing observed in diploids (3), but in fact we have actually never proved it to be so.

It seemed desirable to attempt purification of the *dsdC* activator for a number of reasons. The low constitutive phenotype of $dsdC^c$ (constitutive)/$dsdC^+$ (inducible) heterozygotes in vivo had suggested that the *dsdC* activator, unlike the *araC* repressor-activator (14), lacks repressor function (3). It was not possible to decide this point using *dsdC* product formed in the in vitro system for technical reasons: the amount produced was small, and the range for concentration variation consequently was limited. It was also not possible to uncouple transcription and translation of *dsdA* in an in vitro system that relied on endogenously produced *dsdC* activator; thus we could not define the level at which control was exerted. The *dsdC* activator was also of interest because its action, unlike that of regulatory proteins in other catabolic systems, was to a significant extent independent of the cyclic AMP-cyclic AMP binding protein (cAMP-CAP) system (10). In this communication we describe a partial purification of the D-serine deaminase activator and present evidence that its role is that of a strictly positive transcriptional agent.

MATERIALS AND METHODS

Chemicals. Rifampin and chloramphenicol were purchased from Sigma, ribonuclease-free deoxyribonuclease (DNase) I from Worthington. Diethylaminoethyl (DEAE)-cellulose (0.91 meq/g) was from Schleicher and Schuell Co., Keene, N.H.; Munktell 410 cellulose was from Bio-Rad; and highly polymerized, double-stranded calf thymus DNA came from Worthington.

Bacteria and bacteriophages. The bacterial strains used in this study are described in Table 1.

Preparation of S-30 extracts. S-30 extracts were prepared from strain TE6000/pKB252 as described previously and calibrated for optimal conditions in the cell-free system by measuring λ*plac*5 (17) DNA-directed β-galactosidase synthesis (8).

Preparation of DNA. DNA was prepared as described (8).

Conditions for cell-free protein synthesis. Conditions for our standard DNA-directed coupled transcription-translation system were according to Zubay (18) with some modifications (8). Unless otherwise stated, template DNA was always from strain EM6177, $dsdC^+$ $dsdO^+$ $dsdA^+$, and was present at a concentration of 50 μg/ml of synthesis mixture. All the necessary ingredients and template DNA were incubated at 36°C

for 3 min before addition of S-30 extract (6.5 to 9.0 mg of protein per ml of synthesis mixture) to initiate protein synthesis. Incubation with shaking was continued at 36°C for 40 min for D-serine deaminase synthesis. Incubation for λp*lac*5 DNA-directed β-galactosidase synthesis was at 36°C for 60 min. The reactions were stopped by chilling on ice.

Assays for enzyme activity. Assay procedures for measuring D-serine deaminase and β-galactosidase activities were described previously (8). D-Serine deaminase activity is expressed as nanomoles of pyruvate formed from D-serine in 20 min per milliliter of synthesis mixture. β-Galactosidase activity is presented as units of absorbancy at 420 nm per milliliter of synthesis mixture per 60-min assay time.

Preparation and purification of *dsdC* extracts. Cells of the appropriate strains were grown at 30°C in the presence of 2 mM magnesium acetate and 1 mM D-serine to mid-log phase as described by Zubay for S-30 extracts (18).

Cells were frozen at −70°C in a Revco ultra-low freezer overnight. The frozen cell paste (50 g) was allowed to soften at 4°C. (All the steps of purification were carried out at 4°C.) Cells were suspended in 200 ml of buffer A [0.01 M tris(hydroxymethyl)aminomethane-acetate, pH 8.2; 0.01 M magnesium acetate; 0.01 M 2-mercaptoethanol; 0.06 M potassium chloride; 5% glycerol; and 1 mM D-serine]. The suspension was centrifuged at 12,000 × *g* for 20 min. The sedimented cells were resuspended in 200 ml of buffer A and centrifuged again as above. The washed cells were resuspended in 65 ml of buffer A and lysed in an Aminco French pressure cell at a pressure of 6,000 lb/in^2. The broken cell suspension was centrifuged at 30,000 × *g* for 30 min, and the supernatant was then centrifuged at 100,000 × *g* (in a Spinco 30 rotor) for 3.5 h. The resulting supernatant was dialyzed overnight against 2 × 1 liter of buffer B (0.01 M potassium phosphate, pH 7.7; 0.7 mM dithiothreitol; 5% glycerol; and 1 mM D-serine), then immediately chromatographed on a DEAE-cellulose column.

dsdC extracts were dialyzed against buffer C [0.01 M tris(hydroxymethyl)aminomethane-acetate, pH 8.2; 0.014 M magnesium acetate; 0.06 M potassium acetate; 0.7 mM dithiothreitol; 5% glycerol; and 1 mM D-serine] for assaying in the cell-free system.

DNA-cellulose columns were prepared according to the method of Alberts and Herrick (1) and were equilibrated with buffer D (0.01 M potassium phosphate, pH 7.2; 0.5 mM dithiothreitol; 5% glycerol; and 1 mM D-serine).

Details of chromatography on DEAE-cellulose and DNA-cellulose columns are described in the legend to Fig. 1.

RESULTS

Partial purification of *dsd* activator. We assumed that the *dsdC* activator, like the *araC* product (14), was an unstable DNA-binding protein. We also expected to find only a few molecules per wild-type *E. coli* K-12 cell; the D-serine deaminase in vitro system, however, should provide an adequate assay.

Therefore, we attempted a purification procedure similar to that of Yang and Zubay's (17) successful protocol for the *araC* protein (Fig. 1). We used strain EM1607 (*dsdC*$^+$, nonreverting *dsdA*) as source of *dsdC* activator. Strain EM6116, which is deleted for the entire *dsd* region and should therefore produce no *dsdC* activator, was used as control. After separation of membranes and ribosomes from cell lysates by high-speed centrifugation, proteins and nucleic acids were absorbed on a DEAE-cellulose column, and removal of nucleic acids was achieved by selective desorption of proteins from the column (crude DEAE fraction). Subsequent DNA-cellulose column chromatography (1) resulted in further elimination of those proteins which did not bind to DNA. We suspected that the *dsd* activator might prove especially unstable in the absence of its ligand, and so we in-

TABLE 1. *Bacterial strains*

Strain no.	Purpose	Relevant genotype	Reference
TE6000/ pKB252	Source of S-30 extract	Δ(*dsdC-dsdA*) Δ*lac*/ColE1-*tet*$^+$-λcI$^+$	Heincz et al. (8)
EM6177	Source of *dsd* template DNA	Δ(*dsdC-dsdA*)/λ*dsdC*$^+$*O*$^+$*A*$^+$	Palchaudhuri et al. (11)
EM61606	Source of *dsd* template DNA	Δ(*dsdC-dsdA*)/λ*dsdC*$^+$*O6A*$^+$	[a]
EM1607	Source of *dsdC*$^+$ extract	*dsdC*$^+$ *O6A6*	Palchaudhuri et al. (11)
EM6116	Source of *dsdC* extract	Δ(*dsdC-dsdA*)	Palchaudhuri et al. (11)
EM1600		*dsdC*$^+$ *dsdO6 dsdA*$^+$	McFall (10)

[a] The lysogen EM61606 was constructed as follows. Strain EM6116 was transduced to *dsdA*$^+$ with P1 phage grown on strain EM1600. A culture of a *dsdO6 dsdA*$^+$ transductant was exposed to λd*dsdC*$^+$*dsdO*$^+$*dsdA*$^+$ and λ*y*199 (*cI857Sam7xis6b515b519*) phage at multiplicities of 5 each. About half of the lysogens formed harbor the λd*dsd* phage; the lysogens can be recognized because they yield both λ*y*199 and λd*dsd* phage on thermal induction (11). A significant proportion yield λd*dsdO6* phages because of crossover events. Phage from one such lysogen were used to transduce strain EM6116 to *dsdA*$^+$, as described for construction of strain EM6177 (11), with *y*199 again as helper. A transductant, denoted EM61606, was obtained which yielded λ*dsdO6dsdA*$^+$ and λ*y*199 phages at a ratio of about 10^4:1 and with an average burst size of 20.

Purification of *dsdC* regulatory protein

Breaking of washed cells in a French pressure cell, 6,000 lb/in^2

↓

100,000 × *g* supernatant (S-100 extract)

↓

DEAE-cellulose column chromatography: 0 to 0.25 M NaCl fraction, saturated $(NH_4)_2SO_4$ precipitation, centrifugation, dialysis (crude DEAE fraction, 15 mg of protein per ml)

↓

DNA-cellulose column chromatography of 6.5-ml portions of crude DEAE fraction: 0.3 to 0.5 M NaCl fraction, dialysis (partially purified *dsdC* fraction, 200 μg of protein per ml)

FIG. 1. *The two major steps of purification are the DEAE-cellulose and DNA-cellulose column chromatography.* ***Step 1.*** *The dialyzed supernatant of the 100,000 × g centrifugation was passed through a DEAE-cellulose column (4.5 by 30 cm) previously equilibrated with buffer B. After the application of the sample, the column was washed with 2 liters of buffer B and eluted with 0.25 M NaCl in buffer B. The protein-containing fractions were pooled and adjusted to 67% saturation by the addition of saturated ammonium sulfate solution to precipitate the bulk of proteins. The precipitate was collected by a 10-min centrifugation at 12,000 × g. The precipitate was dissolved in 30 ml of buffer C, dialyzed against 2 × 1 liter of buffer C overnight, then frozen and stored in nitrocellulose tubes in liquid N_2. This preparation was regarded as the crude DEAE fraction, which contained 15 mg of protein per ml.* ***Step 2.*** *Portions of the crude DEAE fraction (6.5 ml) were dialyzed against 3 × 1 liter of buffer D for 3 h. They were immediately applied to a DNA-cellulose column (0.8 by 10 cm). The column was then washed with buffer D to remove the weakly adsorbed proteins until the absorbancy at 280 nm of the eluate was less than 0.05. Stepwise elution of the DNA-bound proteins was done by increasing the concentration of NaCl in buffer D to 0.1 M, 0.2 M, 0.3 M, and 0.5 M. Most of the activator activity was present in the fraction that eluted between 0.3 and 0.5 M NaCl. This fraction was designated as partially purified dsdC$^+$ protein; it was dialyzed against 2 × 1 liter of buffer C overnight, frozen, and stored in nitrocellulose tubes in liquid N_2. This preparation contained 200 μg of protein per ml in a 2.4-ml final volume. Preparation of ΔdsdC extract was done as described above. The final product that eluted from the DNA-cellulose column between 0.3 and 0.5 M NaCl contained 170 μg of protein per ml in a 2.5-ml final volume.*

cluded 1 mM D-serine in all buffers used throughout the purification. All procedures were carried out as quickly as possible. The crude DEAE fraction and the various DNA-cellulose column fractions were stored in liquid nitrogen. For the DNA-cellulose step, 6.5-ml portions of the crude DEAE fraction were applied to the column and eluted with increasing concentrations of NaCl, up to 0.5 M. The crude DEAE and the various DNA-cellulose fractions were then tested for ability to activate D-serine deaminase synthesis at 36°C from a *dsdC*$^+$ *dsdO*$^+$ *dsdA*$^+$ template in the cell-free system. Although the *dsdC*$^+$ gene is present on this DNA, it is not expressed at 36°C (8). The results are presented in Fig. 2.

It may be seen that significant *dsdC* activator was present in the crude DEAE fraction from the *dsdC*$^+$ strain, which gave a four- to fivefold stimulation of D-serine deaminase synthesis per unit volume. About 80% of this activity was recovered in the 0.3 to 0.5 M NaCl fraction from the DNA-cellulose column, which gave about 20-fold stimulation per unit volume. There was weak but detectable activity in the other NaCl fractions from the DNA-cellulose column; we suspect that part of this earlier elution may have been due to overloading of the column. No activity was found in the corresponding fractions from the *dsd* deletion strain. If we define a doubling of D-serine deaminase activity as a specific activity unit for *dsdC* activator, then the crude DEAE extract has 0.3 such units per mg of protein per ml of synthesis mixture. The

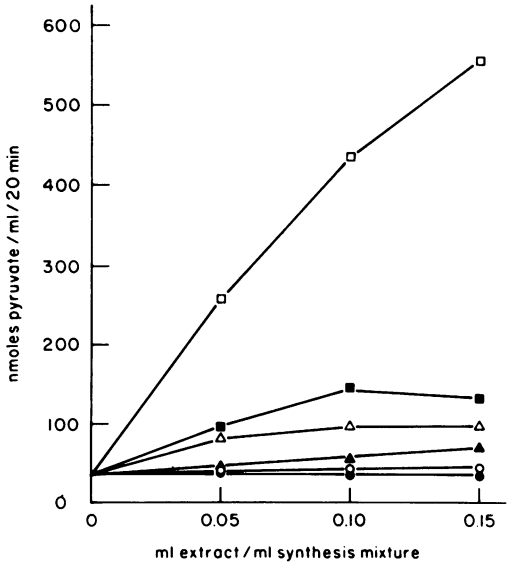

FIG. 2. *D-Serine deaminase synthesis as a function of added dsdC extracts. Synthesis was done as described in the text for the coupled system. Each synthesis mixture contained 0.5 mM D-serine. (■) Extract prepared from strain EM1607 (dsdC$^+$) by DEAE-cellulose column chromatography; (○) 0.1 M NaCl eluate of EM1607 extract from the DNA-cellulose column; (▲) 0.2 M NaCl eluate; (△) 0.3 M NaCl eluate; and (□) 0.5 M NaCl eluate; (●) extract prepared from strain EM6116 (ΔdsdC) eluted from the DNA-cellulose column between 0.3 to 0.5 M NaCl.*

partially purified fraction has 100 such units per mg of protein per ml of synthesis mixture, representing a 330-fold increase in the DNA-cellulose step. It should be noted, however, that we cannot necessarily equate this activity increase with degree of purification, as we do not know the level of activity in the pre-DEAE material, nor do we have any quantitative information on the activation process. Both the crude DEAE fractions and the partially purified 0.3 to 0.5 mM NaCl fraction from the DNA-cellulose column inhibited β-galactosidase synthesis programmed by λp*lac*5 DNA. The extent of the inhibition was to 45 and 17% of maximal levels, respectively (data not shown). This inhibition was reversed by the presence of isopropyl-β-D-thiogalactopyranoside (1 mM) to 70% of maximal level. Strain EM1607 is *lac*$^+$, and we apparently partially copurified *lac* repressor.

Instability of the *dsdC* activator in the absence of D-serine. Because of the possibility that the *dsdC* activator is unstable, we routinely stored it in liquid nitrogen in buffers containing D-serine. We found both the crude DEAE fraction and the partially purified fraction to be stable for weeks under these conditions.

To test the effect of D-serine on stability, we dialyzed portions of each fraction for 18 h against buffer C lacking amino acid. The dialysates were then either tested immediately for ability to activate cell-free D-serine deaminase synthesis, or stored in liquid nitrogen and tested subsequently. One portion of the dialyzed crude DEAE fraction was also subjected to DNA-cellulose column fractionation, and the eluates were tested for ability to activate cell-free D-serine deaminase synthesis (Table 2). It may be seen that there was no immediate loss of *dsdC* activator function simply upon removal of D-serine, except when further fractionation of the preparation was attempted. In that case, no activator activity could be recovered, even though D-serine was subsequently present in the synthesis mixture. Thus, the *dsdC* activator is reasonably stable to routine purification procedures as long as D-serine is present. Moreover, its instability to further purification in the absence of D-serine indicates that D-serine and not a D-serine metabolite is probably its natural ligand.

Lack of repressor function in the *dsdC* activator. Upon titration, we found the D-serine concentration that we used to stabilize the *dsdC* activator (0.5 mM) to be optimal for in vitro induction. A concentration of 0.15 mM was suboptimal; it promoted D-serine deaminase synthesis from the *dsdC*$^+$ *dsdO*$^+$ *dsdA*$^+$ template at a rate about 50% that of the optimal concentration (not shown). Therefore we can ask what effect increasing the concentration of *dsdC* activator, without increasing the suboptimal concentration of D-serine, would have on the rate of D-serine deaminase synthesis. If the *dsdC* activator has repressor as well as activator function, we would expect the rate of D-serine deaminase synthesis to decrease.

The results of an experiment which titrates the response of D-serine deaminase synthesis to *dsdC* activator concentration at constant final (0.15 mM) D-serine concentration is presented in Fig. 3. It may be seen that there was no repression of D-serine deaminase synthesis as *dsdC* activator concentration increased. In fact, the rate of synthesis rose. The increase in synthesis at suboptimal D-serine concentration presumably reflects a shift of D-serine (DS) toward the bound state in an equilibrium k = (DS)(activator)/(DS·activator); as (activator) increases, so does (DS·activator).

TABLE 2. *Stability of dsdC*$^+$ *activator preparations in the absence of* D-*serine*[a]

dsdC$^+$ activator preparation	Incubation system	Dsdase activity (nmol of pyruvate/ml per 20-min incubation with DS)
Crude DEAE fraction	Complete	68
	Complete + 0.5 mM DS	143
Crude DEAE fraction, dialyzed against buffer C without DS	Complete	37
	Complete + 0.5 mM DS	141
Partially purified fraction	Complete	326
	Complete + 0.5 mM DS	554
Partially purified fraction, dialyzed against buffer C without DS	Complete	35
	Complete + 0.5 mM DS	592
Partially purified fraction, purified on DNA-cellulose in the absence of DS	Complete	35
	Complete, + 0.5 mM DS	85

[a] The crude DEAE and partially purified *dsdC* activator preparations were prepared as described in the text and Fig. 1. To remove D-serine (DS), the preparations were dialyzed for 18 h against D-serine-free buffer C (two changes of buffer). They were then tested immediately for activator activity in the cell-free system, or, in the last case, after fractionation on DNA-cellulose in the absence of D-serine. The "complete" cell-free D-serine deaminase (Dsdase) incubation system consists of all components except D-serine. A 0.15-ml sample of the respective *dsdC* activator preparation was used for each ml of incubation mixture. Except in the case of the dialyzed fractions, these activator preparations were 1.0 mM in D-serine; thus their complete incubation mixtures contained a basal level of 0.15 mM D-serine.

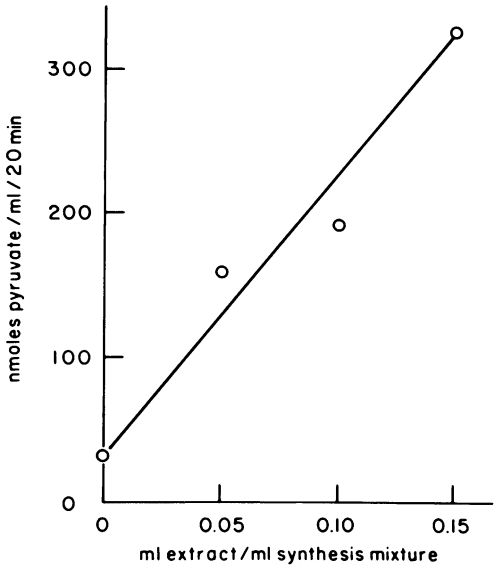

FIG. 3. *Effect of dsdC⁺ protein on D-serine deaminase synthesis with suboptimal D-serine. D-Serine deaminase synthesis was done as described in the legend to Fig. 2 except that D-serine was present at a final concentration of 0.15 mM in each synthesis mixture. The extract used was the 0.3 to 0.5 M NaCl eluate of the EM1607 extract from the DNA-cellulose column. It contained 200 μg of protein per ml.*

Uncoupling of transcription and translation in the D-serine deaminase in vitro system. Our previous work suggested that the *dsdC* activator exerted its effect at transcription. Thus, the activator–D-serine complex can partially satisfy the cAMP-CAP requirement for enzyme synthesis (10). Since cAMP-CAP complex acts at transcription in other systems, it likely also does so for D-serine deaminase.

To examine this point directly, we developed a DNA-directed uncoupled in vitro system for the synthesis of D-serine deaminase similar to that of Wild et al. (15). Translation was blocked by omission of amino acids from the synthesis mixture, while transcription was allowed to proceed. Initiation of transcription was then blocked by rifampin, and the system was recoupled by addition of amino acids to allow translation. For these experiments the system was programmed with *dsdC⁺ dsdO6* (operator constitutive) *dsdA⁺* DNA from strain EM61606, to eliminate the *dsdC* activator–D-serine requirement. During transcription no detectable D-serine deaminase synthesis occurred under the above conditions, as shown by controls in which no amino acids were added with the rifampin (data not shown).

When transcription was allowed to proceed for varying lengths of time, followed by 30 min with rifampin and amino acids to complete transcription and translation of the message formed, it was found that at least 3 min was necessary for the appearance of the first detectable *dsd* transcripts (Fig. 4A). The rate of transcription of *dsd* mRNA increased linearly for an additional period of 12 min and decreased after 20 min. Translation of completed *dsd* transcripts began at 3 min; the rate increased linearly for an additional 7 min (Fig. 4B).

The time course of D-serine deaminase synthesis under standard conditions (coupled synthesis) is shown in Fig. 4C. The maximal level of synthesis of the enzyme in the uncoupled system (Fig. 4B) reached about 70% of that in a coupled system.

The level of action of *dsdC⁺* activator. Under optimal conditions for the uncoupled system as described above, it was possible to ask whether the *dsdC⁺* activator exerts its control at the level of transcription or translation in DNA-directed cell-free synthesis of D-serine deaminase. The complete coupled system was used as a control. During 40 min of incubation at 36°C, the activator stimulated D-serine deaminase synthesis 20-fold (Table 3). Three controls were used in the uncoupled system. In the first control, activator was present during transcription, but after addition of rifampin and amino acids the reaction was stopped; in the second, activator was also present during transcription, but instead of amino acids water was added for translation; in the third, activator was present only during translation (Table 3). In all cases, enzyme synthesis was only slightly higher than the basal level produced in the coupled system (without activator).

When *dsdC⁺* protein was added to the synthesis mixture at the initiation of transcription, and 15 min later further transcription was blocked by rifampin and the system was recoupled with amino acids, D-serine deaminase synthesis proceeded at about 80% of the level in the coupled system (Table 3). These data show that unless the *dsdC⁺* activator is present during transcription practically no synthesis of the enzyme occurs. Thus the activator functions as a transcriptional control element.

Kinetics of syntheses of functional *dsd* mRNA and D-serine deaminase in the DNA-directed cell-free system. Rifampin, DNase, and chloramphenicol were used in the coupled system to examine transcription initiation, chain elongation, and translation of mRNA separately (15) (Fig. 5). The coupled system was programmed with *dsdC⁺ dsdO6 dsdA⁺* DNA and with λ*plac*5 DNA templates. Initiation of *dsd* mRNA began at 3.4 min (Fig. 5A). The first mRNA chains were completed at 7.4 min (rifam-

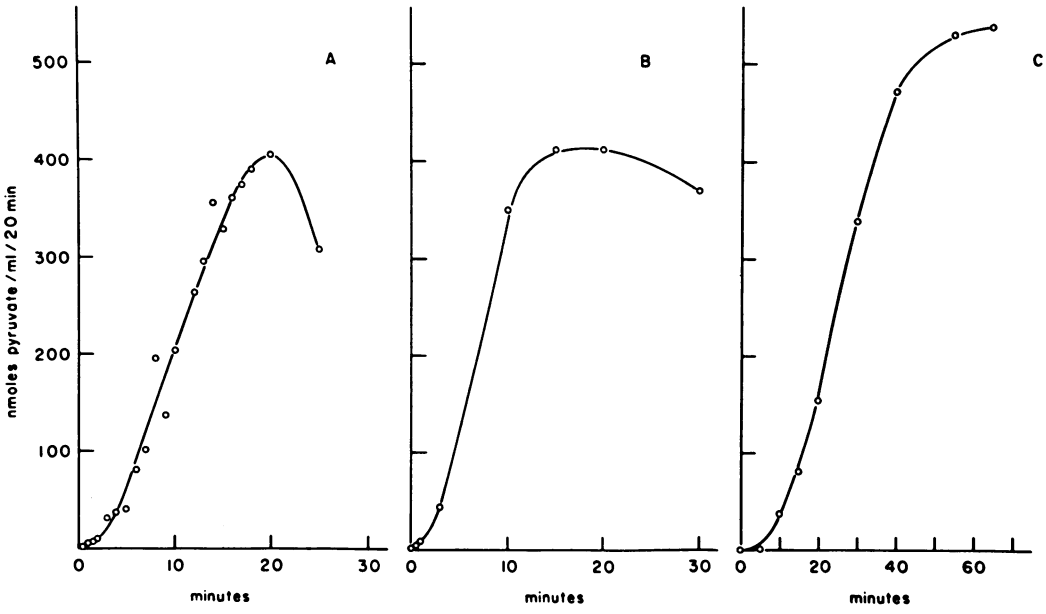

FIG. 4. *DNA-directed uncoupled synthesis of D-serine deaminase in the cell-free system. Standard conditions of enzyme synthesis were used, except as indicated below or in the text. Template DNA was prepared from strain EM61606, dsdC⁺ dsdO6 dsdA⁺, and was present at a concentration of 54 μg/ml. (A) Time dependence of dsd mRNA initiation. Amino acids (0.22 mM) were omitted from the complete synthesis mixtures and were added together with rifampin (20 μg/ml of synthesis mixture) at intervals as indicated. Incubation was continued at 36°C for 30 min to allow translation. (B) Time course of dsd mRNA translation. Uncoupled transcription was terminated after 20 min of incubation when rifampin and amino acids were added as above. Translation was allowed to proceed for varying lengths of time. (C) Coupled synthesis of D-serine deaminase under standard conditions.*

TABLE 3. *Effect of partially purified dsdC⁺ activator on the in vitro coupled and uncoupled synthesis of D-serine deaminase*[a]

Conditions for coupled system[b, c]			Dsdase synthesis (nmol of pyruvate per ml per 20-min incubation with DS)
Complete			30
Complete + *dsdC⁺* activator			600
Conditions for uncoupled system[c, d]			
Present at 0 time	Added at 15 min	Incubation to allow translation (min)	Dsdase synthesis (nmol of pyruvate per ml per 20-min incubation with DS)
dsdC⁺ activator	Rifampin and amino acids	0	90
dsdC⁺ activator	Rifampin and water	30	44
No addition	Rifampin, amino acids, and *dsdC⁺* activator	30	77
dsdC⁺ activator	Rifampin and amino acids	30	480

[a] When partially purified *dsdC⁺* activator was present, 0.15 ml of extract per ml of synthesis mixture was used (200 μg of protein per ml of extract). Dsdase, D-Serine deaminase; DS, D-serine.

[b] D-Serine deaminase synthesis in the coupled system was done under standard conditions.

[c] Synthesis was programmed with 50 μg of *dsdC⁺ dsdO⁺ dsdA⁺* DNA template per ml of synthesis mixture.

[d] Complete incubation mixtures were incubated at 36°C for 15 min to allow transcription. Rifampin was then added to a final concentration of 20 μg/ml of synthesis mixture. Translation was initiated by addition of the 20 amino acids (0.22 mM each). Incubation for translation was at 36°C for 30 min. 0 time is the initiation of incubation at 36°C by the addition of S-30 extract.

pin versus DNase), indicating that about 4 min was necessary to complete a *dsd* message (Fig. 5A). The first appearance of translated *dsd* messages was at 10.2 min. The time interval between completion of *dsd* mRNA and the appearance of D-serine deaminase was 2.8 min (chloram-

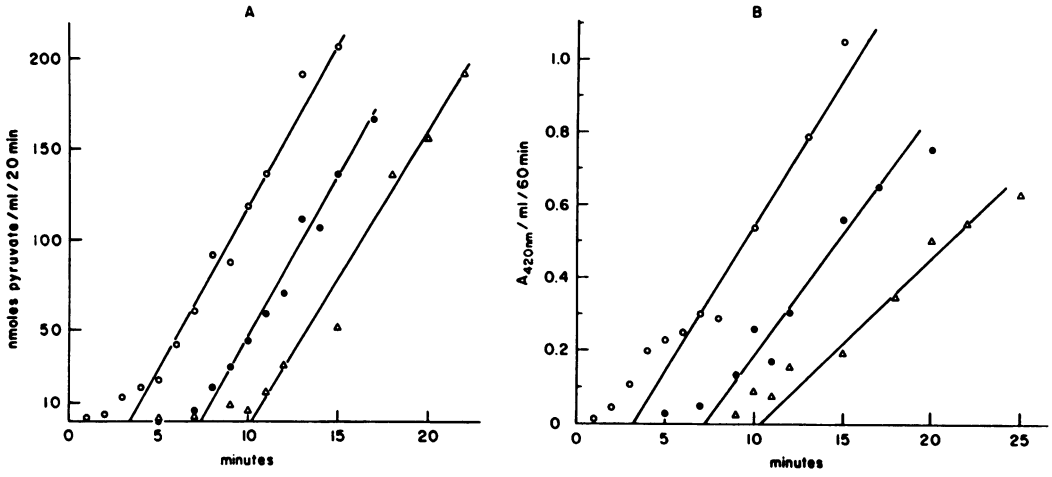

FIG. 5. *Time course of message initiation, elongation, and translation for dsd and lac mRNA's. (A) Synthesis of* D-*serine deaminase was as described in the legend to Fig. 2, using 54 μg of dsdC⁺ dsdO6 dsdA⁺ template DNA per ml. At different times of incubation, rifampin, DNase, and chloramphenicol were added to the synthesis mixtures, and incubation was continued for an additional period of 30 min.* (○) *Rifampin, 5 μg/ml;* (●) *DNase, 5 μg/ml;* (△) *chloramphenicol, 100 μg/ml. (B) β-Galactosidase synthesis was directed by 26 μg of λplac5 DNA per ml. Experimental conditions were as described for A.*

phenicol versus DNase).

When β-galactosidase synthesis was carried out in the same manner, very similar time requirements were observed (Fig. 5B). Transcription was initiated from λ*plac*5 DNA at 3.2 min; the message was completed at 7.2 min; and the first appearance of β-galactosidase was at 10.3 min.

DISCUSSION

The failure of the *dsdC* activator to act as a repressor at suboptimal D-serine concentration indicates that it is solely a positive control element. This result is in accord with previous genetic evidence—mixed dominance in *dsdC*^{c}/*dsdC*$^+$ merodiploids and ease of isolation of initiator constitutive mutants (2, 3)—that suggested absence of a negative control. Positive control is the logical induction mechanism for D-serine deaminase, because of the toxic effect of D-serine on *E. coli* K-12 (9) and the presence of the cAMP-CAP-mediated catabolite repression control. Thus, *dsdC* activator–D-serine complex can partially replace the cAMP-CAP requirement in wild-type cells; otherwise the cells could not grow in the presence of both glucose and D-serine (2). If the *dsdC* activator were strictly a negative element, this "catabolite override" would probably require yet another control element, as in the case of histidine utilization (13). Positive control with an override component is ideal for the D-serine deaminase system, but there is no obvious need for a negative control as well.

We have assumed that the *dsdC* activator is a protein, like other pathway-specific regulatory gene products, although we do not have specific proof such as suppressible amber mutations affecting *dsdC*. The fact that the DEAE purification step, which removes nucleic acids, increases *dsdC* activator activity to a measurable level indicates that the activator is not an RNA. Moreover, its copurification with the *lac* repressor and its role as a DNA-binding element able to interact specifically with the small molecule D-serine indicate that it is most probably a protein.

Happily, the *dsdC* activator seems to be fairly stable. Most of the activity in the crude DEAE fraction was recovered after further purification on DNA-cellulose when D-serine was present during the latter step. Even in its absence the activator is stable for at least several hours at 4°C and for months in liquid nigrogen; readdition of D-serine restores its activity completely. Since the amino acid protects it against inactivation during purification and converts it from the inactive to the active state, it is likely that D-serine and not a derivative is the natural ligand (inducer).

We do not understand why nearly 4 min should be required for transcription of *dsdA* mRNA. This is nearly as long as required for transcription of *lacZ* mRNA under the same conditions. The latter should be more than twice the size of the *dsdA* message, on the basis of the relative monomeric molecular weights of the proteins they encode (135,000[5] versus

45,500[6]). It could be that there is another gene in the *dsd* operon, proximal to *dsdO*, that we are not aware of. However, there is no obvious function for the product of such a gene. The D-serine permease gene *dag* is not linked to *dsdA* (4, 12). D-Serine deaminase, the *dsdA* gene product, is the only activity necessary for D-serine deamination (6). M. Blundell (personal communication) has measured *dsdA* mRNA formed in vivo, and finds two classes. The bulk of it corresponds in size to that expected to code for the D-serine deaminase protein alone, which suggests that there are no other genes in the operon. There is a minor component that is about twice as large and decays more rapidly than the smaller mRNA. It seems likely that this larger mRNA results from readthrough of the *dsdA* message past its normal termination, but it could still be that the synthesis and/or maturation of *dsdA* mRNA is a more complex process than we anticipate.

ACKNOWLEDGMENTS

We are grateful to M. Blundell for communication of unpublished work, and N. E. Kelker, I. Schenkein, and G. Wilcox for very helpful discussions.

This work was supported by Public Health Service research grants GM-11899 to E.M. and GM-06048 to W. K. Maas from the Institute of General Medical Sciences.

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