JOURNAL OF BACTERIOLOGY, Mar. 1975, p. 1078-1084

Vol. 121, No. 3
Printed in U.S.A.

Isolation and Characterization of D-Serine Deaminase Constitutive Mutants by Utilization of D-Serine as Sole Carbon or Nitrogen Source

FREDRIC R. BLOOM[1] AND ELIZABETH McFALL*

Department of Microbiology, New York University School of Medicine, New York, New York 10016

Received for publication 16 December 1974

Mutants constitutive for D-serine deaminase (Dsdase) synthesis were isolated by utilizing D-serine as sole nitrogen or carbon source in the chemostat. This method generated only regulatory constitutive (*dsdC*) mutants. The altered *dsdC* gene product in these strains is apparently able to bind D-serine more efficiently than the wild-type *dsdC*$^+$ gene product—a selective advantage. Constitutive synthesis of Dsdase in all of these *dsdC* mutants is extremely sensitive to catabolite repression, and catabolite repression is reversed by the addition of D-serine. Of the 15 mutants generated by this method, none are suppressible by *supD*, *supE*, or *supF*. Mutations to a low level of constitutivity (maximal specific activity of 9) occur much more frequently than mutations to a high level (maximal specific activity of 79). High level constitutive synthesis of Dsdase results from the synthesis of an altered *dsdC* gene product— not from loss of ability to form the *dsdC* product. Dsdase synthesis is not regulated by the nitrogen supply in the medium, as nitrogen starvation does not result in the derepression of Dsdase synthesis.

D-Serine deaminase (Dsdase) functions primarily as a detoxifying enzyme in *Escherichia coli* K-12 as the inducer. D-Serine (DS) is toxic at concentrations greater than 25 μg/ml (3, 7). Dsdase converts DS to pyruvate and NH_3, allowing DS to serve as the sole source of carbon and nitrogen. Dsdase thus functions secondarily as a catabolic enzyme. The induced synthesis of Dsdase is subject to catabolite repression (CR); efficient induction requires cyclic adenosine 5′-monophosphate (cAMP) and the cAMP binding protein (CAP factor) as well as DS (11). The DS-*dsdC* regulatory gene product complex can partially replace the cAMP-CAP factor complex in promoting Dsdase synthesis (11).

Six mutants constitutive for Dsdase synthesis were isolated from a *dsdC*$^+$ strain by utilization of DS as sole carbon source limiting growth in the chemostat and by alternate subculture in media with DS or NH_3 as sole nitrogen source. These were mapped in two regions, *dsdC* and *dsdO*, close to the structural gene for Dsdase (*dsdA*) (7, 12). Previously isolated *dsdC* mutants (two) synthesize Dsdase at a low constitutive rate (7). Constitutive Dsdase synthesis is extremely sensitive to CR in these mutants and is hyperinducible in one (8) but not the other. The *dsdC*$^+$ gene has a partial *trans* effect on constitutive synthesis in these dsdC strains, and the *dsdC* region is thought to elaborate a regulatory product (10).

Three of the other four constitutive mutants form Dsdase at a high rate in the absence of DS; the other becomes progressively more constitutive as the growth temperature rises (8, 10). Dsdase synthesis is completely resistant to CR in these strains (11), and the mutations are fully *cis* dominant (10). These mutations were denoted *dsdO* (11). *dsdO* is the site of response to induction and CR and is thought to represent an operator-initiator region for Dsdase (11).

The nature of the regulation of Dsdase is of interest, as most catabolic enzymes do not have another function. To learn more about the nature of Dsdase, we attempted to isolate more constitutive mutants. Three methods were used and each yielded a different spectrum of mutants. One method involved plating the low constitutive, catabolite-sensitive nonhyperinducible *dsdC* mutant on solid medium with glucose as the sole carbon source and DS as the sole nitrogen source. This method generated secondary mutants in the *dsdO* region by selecting for escape of Dsdase synthesis from CR. These mutants will be described in a later paper. Another method utilized the chemostat with DS as limiting carbon or nitrogen source

[1] Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

and generated *dsdC* mutants. This paper will deal primarily with the isolation and characterization of such *dsdC* mutants, and particularly with two of them, strains FB5019 and FB5020. These two mutants synthesize Dsdase at a very high constitutive rate as a result of an altered *dsdC* gene product. The third method is the previously described alternate subculture (7), which yields both *dsdO* and *dsdC* mutants.

(This work was presented in partial fulfillment of the requirements for the Ph.D. degree in Basic Medical Sciences, New York University. A preliminary report was presented at the 73rd Annual Meeting of the American Society for Microbiology 1972.)

MATERIALS AND METHODS

Bacterial strains. The strains utilized in this study are described in Table 1.

Chemicals. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was purchased from K and K Laboratories, and DS was purchased from Nutritional Biochemicals Corp.

Media. The minimal medium used in this study has been described previously (12). N-free medium is minimal medium without $(NH_4)_2SO_4$ and NH_4Cl. Nutritional requirements were added at 50 μg/ml. Carbon sources such as glucose were added at a final concentration of 1%, and the medium was designated as glucose minimal medium or glucose N-free medium.

TABLE 1. *Bacterial strains*[a]

Strain no.	Relevant genotype[b]	Parent
K140	*met ura sup*$^+$ HfrC	
KL16-99	*recA str*$^+$ *dsdA*$^+$ *dsdC*$^+$	
K37	*supD dsdA*$^+$ *dsdC*$^+$	
C600	*supE dsdA*$^+$ *dsdC*$^+$	
H12R8A	*supF dsdA*$^+$ *dsdC*$^+$	
FB5019	*dsdC19 met ura sup*$^+$	K140
FB5020	*dsdC20 met ura sup*$^+$	K140
FB5025	*dsdC25 met ura sup*$^+$	K140
EM1104	*dsdA dsdO1 aroC purF*	W3828
EM1200	*dsdO2 lac str*	W3828
EM1400	*dsdC4 lac str*	W3828
EM1600	*dsdO6 lac str*	W3828
EM1900	*dsdC9 lac str*	W3828
EM1605[c]	*dsdA 10(ts) dsdC*$^+$ *thyA str*	EM1610
W3828	*dsdA*$^+$ *dsdC*$^+$	

[a] J. R. Scott kindly supplied strains K140 and C600, B. Low supplied strain KL16-99, J. Speyer supplied strain K37, A. Garen supplied strain H12R8A, and P. H. A. Sneath supplied strain W3828.

[b] Abbreviations: *dsd*, D-serine deaminase; *met*, methionine; *ura*, uracil; *sup*, suppressor; *lac*, lactose utilization; *str*, streptomycin; *thy*, thymine; ts, temperature sensitivity.

[c] EM1605 is an *aroC*$^+$ *dsdO*$^+$ transductant derived from EM1610 (12).

LBT broth is LB broth (6) to which 50 μg of thymidine per ml has been added. Plates were made by adding agar (Difco) to a final concentration of 15 g/liter to the designated media.

Conditions of growth. All experiments were conducted at 37 C with aeration in a gyratory shaker model G-76 (New Brunswick Scientific Co.). The optical density of the culture was monitored at 660 nm with a Zeiss spectrophotometer.

MNNG mutagenesis. Cultures were mutagenized by the method of Adelberg et al. (1).

P1 mediated transductions. The methods used to prepare P1 lysates and to transduce the *dsd* region were those of Luria et al. (6).

Assay of Dsdase. The procedure for the assay of Dsdase has been described previously (7, 9, 12).

Continuous culture of bacteria. The chemostat apparatus consists of a 2-liter reservoir containing the sterile medium used in the experiment and a Holter microinfusion roller pump (Model RL175) which was used to deliver fresh medium into the flask and to remove equal volumes of culture. Experiments were started late in the afternoon so that the culture could come to a steady state overnight. Samples for enzyme determinations were obtained directly from the flask.

The theory explaining the behavior of cultures in the chemostat was first advanced by Novick and Szilard (15). Briefly, an essential nutrient is made rate limiting for growth by reducing its concentration in the reservoir. Therefore, the rate at which this nutrient flows into the culture will determine the generation time of the culture. Thus the flow rate (f) and the volume (vol) of the culture determine the dilution rate (D) as follows: $D = f/\text{vol}$. At steady state conditions, D becomes equal to the growth rate (u). The mass doubling time of the culture is g = liters/u.

In the "mixing experiments," the two constitutive strains were grown overnight in glucose minimal medium. Four milliliters of each culture was mixed with 42 ml of N-free medium containing glucose as carbon source and 100 μg of DS per ml as nitrogen source. The culture was then placed in the chemostat. Samples from the flask were taken at intervals and streaked for single colonies on LBT plates. Single colonies were suspended in 0.3 ml of assay buffer for assay.

Isolation of a recA derivative of strain FB5020. A *thy* derivative of strain FB5020-1 was prepared by selection for trimethoprim resistance (17). This strain was then mated with strain KL16-99 (*recA str*^{s}), with selection for *thy*$^+$ *str*^{r} recombinants. *recA* is closely linked to *thy*$^+$, and the *recA* marker (18) appeared in several of the *thy*$^+$ recombinants. One of these, denoted FB5020-3, was utilized for a dominance test.

Construction of strains for dominance experiments. The *dsdC* markers *5011*, *5015*, *5019*, and *5020* were transduced by P1 phage from strains FB5011, FB5015, FB5019, and FB5020 into strain EM1104 (*dsdA1 aroC purF*), by selection for *dsdA*$^+$. *dsdC*, *dsdA*$^+$, *aroC*, *purF* transductants were purified and characterized and denoted FB5011-1, FB5015-1, FB5019-1, and FB5020-1, respectively. The F32 episome (*dsdC*$^+$ *dsdA*$^+$ *aroC*$^+$ *purF*$^+$) was introduced into these haploid strains and maintained as

described previously (10). The merodiploid strains were repurified on minimal plates. Two cycles of purification were sufficient to stabilize the merodiploids, except in the case of strain FB5020-1. F32 was not readily stabilized in this strain. Strain FB5020-1 was therefore made *recA*, as described above, and its *recA* derivative, FB5020-3, did form a stable merodiploid with F32. After removal of samples from merodiploid cultures for Dsdase assay, the cultures were streaked on minimal plates, and 10 to 20 single colonies were analyzed for the presence of both *dsdC* and *dsdC*$^+$ markers, as described previously (10).

Detection of suppressors. Presence of *supD*, *supE*, or *supF* in K140 derivatives transduced to *ura*$^+$ by P1 phage grown on strains K37, C600, or H12R8A was demonstrated by spotting loopfuls of a set of T4 amber mutant phage, titer of about 10^8/ml, on lawns of the transductants spread on LBT plates. When a suppressor gene was present, the phage could replicate and caused lysis. The T4 phage were kindly provided by J. Speyer.

RESULTS

Isolation of constitutive mutants in the chemostat. McFall (7) had utilized the chemostat with DS as limiting carbon source to generate the low constitutive mutant EM1400 (*dsdC4*) from strain W3828. An attempt was made to generate more constitutive mutants in the chemostat under a variety of conditions from strains K140 and W3828 to determine whether a wider range of constitutive mutant types than previously observed could be isolated. Strain K140 contains a *ura* mutation suppressible by *supD*, *supE*, and *supF*, which made it possible to test constitutive mutants isolated in this strain for suppressibility.

DS (at a concentration of 100 μg/ml) as sole nitrogen source can be used as the growth-limiting nutrient of a chemostat culture. An MNNG-treated overnight culture of strain K140 or W3828 was therefore placed in the chemostat with N-free medium containing glucose as carbon source and 100 μg of DS per ml as nitrogen source. The generation time was set at approximately 2 h. Constitutive mutants generally overgrew the parental strain in 3 to 4 days under this condition. This procedure generated 12 constitutive mutants—8 from strain K140 and 4 from strain W3828 (Table 2). Three constitutive mutants were also isolated from strain K140 by utilizing 150 μg of DS per ml as the sole carbon source, and two mutants were isolated by utilizing glycerol as carbon source and 200 μg of DS per ml as sole nitrogen source (Table 2), all after MNNG treatment.

A specific attempt was made to generate high rather than low constitutive mutants by decreasing the induced specific activity of Dsdase in the chemostat culture. We sought to operate the chemostat at very fast generation times (<2 h), and so we used glucose instead of glycerol as the carbon source. In one experiment, O-methylserine, a competitive inhibitor of Dsdase (4), was included in the reservoir at a concentration of 600 μg/ml. In another experiment, the chemostat was operated at an incubation temperature of 42 C rather than 37 C (Table 2). The results of mixing experiments will show that these attempts were unnecessary, as high constitutive mutants, if present, will always overgrow the culture.

TABLE 2. *Dsdase constitutive derivatives of K140 and W3828*

Parental strain	Constitutive derivative	Conditions of isolation[a]
K140[b]	FB5011, FB5014, FB5015	150 μg of DS/ml as sole carbon source[c]
	FB5016, FB5017	Glycerol as carbon source and 200 μg of DS/ml as sole nitrogen source
	FB5018, FB5019, FB5020, FB5021, FB5022, FB5023, FB5024, FB5025	Glucose as carbon source and 100 μg of DS/ml as sole nitrogen source
	FB5026	Glucose as carbon source, 100 μg of DS/ml as sole nitrogen source, and an incubation temperature of 42 C
	FB5027	Glucose as carbon source, 100 μg of DS/ml sole nitrogen source, and 600 μg of O-methylserine/ml
W3828	FB5028, FB5029, FB5030, FB5031	Glucose as carbon source and 100 μg of DS/ml as sole nitrogen source

[a] The medium in the reservoir consisted of N-free salts plus the additions as indicated.

[b] All chemostat runs involving K140 required the addition of uracil and methionine to the medium in the reservoir.

[c] The medium in the reservoir contained minimal salts rather than N-free salts.

Dsdase synthesis in constitutive strains. All four mutants derived from strain W3828 and thirteen of fifteen mutants derived from strain K140 synthesize Dsdase at a low constitutive rate similar to that of strain EM1400 (7). Two very high constitutive derivatives of strain K140, FB5019 and FB5020, were also isolated. The synthesis of Dsdase in these mutants, FB5019 and FB5020, occurs at differential (very high constitutive) rates of 49 and 54 when these strains are grown in glycerol minimal medium (Table 3). In repeated experiments, strain FB5020 consistently showed a 10 to 20% higher rate of constitutive synthesis than strain FB5019. Dsdase synthesis in both mutants is extremely sensitive to CR, with synthesis reduced more than 90% in the presence of glucose. Dsdase synthesis is hyperinducible by DS in glucose-grown cultures of the mutants, however (Table 3). Strains FB5019 and FB5020 thus resemble strain EM1400 (2) as well as other low constitutive (*dsdC*) mutants isolated in the chemostat in that Dsdase synthesis in these mutants is sensitive to CR and hyperinducible for constitutive Dsdase synthesis. Dominance experiments similar to those described previously (10) were performed on strains FB5019 and FB5020 and two of the low constitutive mutants, FB5011 and FB5015. They contain mutations in the *dsdC* region (Table 4), as $dsdC^+$ exerts a partial *trans* effect on constitutive synthesis in merodiploids containing the *dsd* regions from these strains. Constitutive Dsdase synthesis is reduced 85 to 90% in the merodiploid as compared to the haploid strains.

TABLE 3. *Dsdase synthesis in FB5019 and FB5020*[a]

Strain	Carbon source	Dsdase sp act
FB5019	Glycerol	49.0
FB5019	Glucose	3.3
FB5019	Glucose + 50 μg of DS/ml[b]	26.0
FB5020	Glycerol	54.0
FB5020	Glucose	4.4
FB5020	Glucose + 50 μg of DS/ml[b]	40.3
K140	Glycerol	0.05
K140	Glucose	20.0
K140	Glucose + 50 μg of DS/ml[b]	6.4

[a] The strains were cultivated to a cell density of 5×10^8/ml in glycerol or glucose minimal medium containing uracil and methionine.

[b] In these experiments, DS at a final concentration of 50 μg/ml was added to cultures of FB5019, FB5020, and K140 growing in glucose minimal medium. Samples were taken at intervals and assayed for Dsdase.

TABLE 4. *Partial trans dominance of $dsdC^+$ in dsdC/$dsdC^+$ merodiploids*[a]

Strain	Relevant genotype	Dsdase sp act
FB5011-1	*dsdA⁺ dsdC11*	6
FB5011-1/F32	*dsdA⁺ dsdC11/dsdA⁺ dsdC⁺*	1.1
FB5015-1	*dsdA⁺ dsdC15*	6
FB5015-1/F32	*dsdA⁺ dsdC15/dsdA⁺ dsdC⁺*	0.8
FB5019-1	*dsdA⁺ dsdC19*	25.3
FB5019-1/F32	*dsdA⁺ dsdC19/dsdA⁺ dsdC⁺*	2.9
FB5020-3	*dsdA⁺ dsdC20 recA*	12.1[b]
FB5020-3/F32	*dsdA⁺ dsdC20 recA/dsdA⁺ dsdC⁺*	1.7
K140	*dsdA⁺ dsdC⁺*	0.05

[a] The haploid and merodiploid strains were cultivated in minimal medium to a cell density of 5×10^8/ml, with requirements added in the case of the haploid strains. Samples were taken from time to time for assay of constitutive Dsdase synthesis.

[b] The introduction of the *recA* allele resulted in a greater than 50% decrease in the constitutive rate of Dsdase synthesis. The reason for this is not understood. It does not appear to be a catabolite effect, as it is not relieved by cAMP.

A detailed analysis of these and other dominance experiments will be presented in a later publication. For the moment, we can state that strains FB5019 and FB5020 and probably all of the other chemostat mutants contain mutations in the *dsdC* region based on the dominance experiments as well as the phenotypic characteristics.

Mapping of the dsdC20 mutation. The *dsdC* region in *E. coli* is 95% linked to *dsdA* (12). To map the mutation in strains FB5020, EM1605 (*dsdA10*(ts) $dsdC^+$ *thyA*) was transduced to $dsdA^+$ using a P1 lysate grown on strain FB5020. The *dsdA10*(ts) mutation specifies a temperature-sensitive Dsdase, and the strain is Dsdase negative at 39 C. The transduction mixture was plated on DS thymine plates, and the plates were incubated at 39 C. One hundred $dsdA^+$ transductants were analyzed, of which 96 were constitutive for Dsdase. Four of the transductants were not constitutive and on repurification were shown to be inducible for Dsdase synthesis. The mutation in strain FB5020 leading to the high constitutive synthesis of Dsdase is 96% linked to *dsdA*, which is consistent with a location in the *dsdC* region.

Suppressibility of dsdC mutations. K140 contains a *ura* mutation which is suppressed by any of the amber suppressors, *supD*, *supE*, or *supF*. The fifteen constitutive mutants derived from K140 were transduced to ura^+ using P1 lysates grown on *supD*, *supE*, and *supF* strains K37, C600, and H12R8A, respectively. The presence of the suppressors in ura^+ transductants was confirmed by the use of a set of T4

amber mutants. Constitutive strains containing the suppressors and nonsuppressed, parent strains were grown overnight in LBT broth and assayed for Dsdase. None of the suppressors had any effect on Dsdase synthesis in these constitutive strains.

Mixing experiments. In the negatively controlled *lac* system, fully constitutive *lacI* mutants can be isolated rather easily. In the *ara* system, which displays aspects of positive control, low constitutive mutants occur more frequently than high constitutive mutants (5). In the Dsdase system, mutants of the FB5019 and FB5020 type appear to be rare as only two were isolated. This finding could be explained by (i) the rarity of the mutational event required to produce the high constitutive phenotype or (ii) a selective disadvantage conferred on strains FB5019 and FB5020 as a result of the high level *dsdC* mutation. Mixing experiments were therefore performed in which cultures of strains FB5019 and FB5020 were mixed with a culture of strain FB5025 (a low constitutive *dsdC* mutant). The cultures were cultivated in the chemostat under conditions used to generate constitutive mutants from strain K140. The reservoir contained N-free medium, uracil, and methionine, with glucose as the carbon source and DS (100 μg/ml) as the sole nitrogen source. The generation time of the culture was adjusted to approximately 1.8 h. Samples were taken at intervals from the flask and streaked on LBT plates. Single colonies were then analyzed for their Dsdase phenotype. The results indicate that both strains FB5019 and FB5020 rapidly overgrew strain FB5025 (Table 5). The selective advantage of the high constitutive mutants indicates that our inability to generate more than two of these mutants is probably due to the rarity of the mutational event leading to the high constitutive phenotype. Therefore the *dsd* system resembles the *ara* system in that high constitutive mutants occur infrequently.

Our inability to generate *dsdO* mutants in the chemostat is probably also due to the relative rarity of *dsdO* mutations. This conclusion is based on analogous mixing experiments, which indicate that *dsdO* mutants have no selective disadvantage when *dsdO* and *dsdC* strains are co-cultivated in the chemostat. Strains EM1400 (*dsdC4*) and EM1600 (*dsdO6*) coexist in the chemostat, and neither strain has a selective advantage (Table 5). In a similar experiment involving strains EM1200 (*dsdO2*) and EM1900 (*dsdC9*), the dsdO mutant seemed to have an advantage.

Effects of nitrogen starvation in FB5020. Prival and Magasanik (16) described experiments which indicate that nitrogen starvation leads to a reversal of CR of histidase synthesis in *K. aerogenes*. Similar experiments in the Dsdase system were undertaken at the suggestion of B. Magasanik. Strain FB5020 was cultivated in the chemostat with N-free medium containing uracil and methionine with glucose as carbon source and 50 mg each of NH_4Cl and $(NH_4)_2SO_4$ per liter as nitrogen source. This concentration of NH_4Cl and $(NH_4)_2SO_4$ assures

TABLE 5. *Mixing experiments utilizing the chemostat*[a]

Strains mixed	Sample (L)	Colonies assayed	FB5019		FB5025		FB5020		FB5025		EM1400		EM1600		EM1200		EM1900	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
FB5019 and FB5025	0	20	12	60	8	40												
	24	20	14	70	6	30												
	48	20	18	90	2	10												
FB5020 and FB5025	0	49					6	12.2	43	87.8								
	48	32					8	25.0	24	75.0								
	60	32					22	68.8	10	31.2								
EM1400 and EM1600	0	56									31	55.4	25	44.6				
	24	20									13	65.0	7	35.0				
	48	20									13	65.0	7	35.0				
	72	20									13	65.0	7	35.0				
EM1200 and EM1900	0	32													4	12.5	28	87.5
	72	32													15	46.9	17	53.1

[a] In each mixing experiment the two strains were grown in the chemostat under conditions used to generate *dsdC* mutant strains. The reservoir contained N-free medium plus glucose as the sole carbon source and 100 μg of DS per ml as the sole nitrogen source. The reservoir also contained uracil and methionine in the experiments involving the K140 derivatives (FB5019, FB5020, and FB5025). The generation time was adjusted to approximately 1.8 h. Samples from the flask were removed at the times indicated and streaked on LBT plates. The plates were incubated overnight at 37 C. Single colonies were picked and were assayed for Dsdase.

conditions of nitrogen limitation (14). When the generation time of the culture was set at 4.7 h, the constitutive specific activity of Dsdase was 1.0. As a control experiment, FB5020 was cultivated in the chemostat with 0.05% glucose minimal medium (condition of carbon limitation) containing uracil and methionine. When the generation time was set at 6.25 h, the specific activity of Dsdase was 78.9. Dsdase synthesis is therefore not derepressed under conditions of nitrogen limitation, although synthesis is derepressed in response to carbon limitation.

Further isolation of mutants by alternate subculture. The alternate subculture method (7) for isolation of Dsdase constitutive mutants is tedious and time consuming, but it yielded two particularly interesting mutants, EM1100 and EM1300. Strain EM1100 (*dsdO1*) harbors a thermosensitive operator mutation, strain EM1300, a low constitutive nonhyperinducible *dsdC* mutation. For this reason we continued to use it, but obtained only three more constitutive mutants from strain W3828 and none from strain K140. Two of the three mutants from strain W3828 appeared to be of the *dsdO* class: like strain EM1600 they were high constitutives, with constitutive Dsdase synthesis insensitive to CR. The other one appeared to be of the *dsdC* class: like strain EM1400 it was low constitutive, hyperinducible, with constitutive Dsdase synthesis very sensitive to CR. These mutants were not characterized further.

DISCUSSION

The method of selecting constitutive mutants in the Dsdase system strongly influences the type of mutants generated. Only hyperinducible *dsdC* mutants were selected in the chemostat, although a number of experimental conditions were employed in an attempt to generate *dsdO* types. The mutant strains selected were probably more capable of utilizing the limited amounts of DS as sole carbon or nitrogen source because the altered *dsdC* gene product was able to bind it more efficiently and so induce Dsdase synthesis more efficiently. It was shown previously (11) that *dsdC* mutants are more sensitive to induction by DS than $dsdC^+$ strains.

The alternate subculture method, on the other hand, selected a preponderance of catabolite-resistant *dsdO* types. The reason is probably the severe catabolite repression imposed at the repeated transfers from media containing NH_3 as nitrogen source to DS as nitrogen source. Once cells reach equilibrium growth in the chemostat, hyperinducible *dsdC* mutants escape this repression. It is surprising that the one nonhyperinducible *dsdC* mutant that we have isolated, EM1300, was selected by alternate subculture, though one would not expect to find such a mutant by the chemostat method either.

dsdO mutants were probably not selected in the chemostat because of their rarity. The mixing experiments (Table 4) demonstrate that *dsdC* and *dsdO* strains can exist in equilibrium in the chemostat under conditions used to select constitutive mutants. Such coexistence suggests that the altered *dsdC* gene product does not confer a selective advantage on either strain EM1400 or EM1900 relative to strain EM1200 or EM1600 and that the *dsdO* mutation does not confer a selective disadvantage on strain EM1200 or EM1600.

The conclusion that the constitutive synthesis of Dsdase in the mutants isolated from the chemostat resulted from mutations in the *dsdC* region is based on the sensitivity of Dsdase synthesis in these strains to CR, the reversal of CR by the addition of DS (8), and on dominance tests. Inducibility ($dsdC^+$) proved partially dominant over constitutivity (*dsdC*) in all five of the chemostat mutants that were tested and in the nonhyperinducible mutant (*dsdC3*) (see above; 10). Two mutants, FB5019 and FB5020, differed from the other strains in that Dsdase synthesis occurred at a very high rather than a low constitutive rate (Table 3). We have no way of knowing whether strain FB5020 is fully constitutive, however, as we have no nonmetabolizable inducer for this system and so cannot determine the maximal rate of Dsdase synthesis. It is clear that low constitutives arise much more commonly than high constitutives.

The *dsdC* mutations in strains FB5019 and FB5020 appear to result in the synthesis of an altered regulatory product rather than in the absence of a regulatory product. The finding that the addition of DS reverses the effects of CR in both strains FB5019 and FB5020 indicates that a regulatory product capable of interacting with DS is still present in these highly constitutive strains. The results of the mixing experiments indicate that although low constitutive DsdC mutants occur more frequently than high constitutive mutants in the chemostat, they are at a selective disadvantage. DsdC mutants thus resemble $AraC^c$ mutants, in which constitutive synthesis of the enzymes of the *ara* operon results from the synthesis of an altered *araC* gene product (5).

The failure to isolate highly constitutive mutants lacking a *dsdC* product led us to suspect that Dsdase synthesis is regulated in a positive manner in that the *dsdC* gene product is re-

quired for the synthesis of Dsdase. If Dsdase were regulated in a negative manner, fully constitutive mutants would result from the absence of a regulatory product and DS would be ineffective in reversing CR. The fact that DS fails to reverse CR only in strains harboring *dsdC3*, a low constitutive *dsdC* mutation that does not allow hyperinducibility (8), is strong evidence for positive control. Further evidence on this point is presented in an accompanying paper (2).

Finally, Dsdase synthesis is not derepressed in the chemostat under conditions of nitrogen limitation, although synthesis is derepressed under conditions of carbon limitation. Nitrogen starvation is evidently an insufficient condition for the relief from CR of constitutive Dsdase synthesis in *E. coli*. In contrast, nitrogen limitation results in relief from CR of histidase and proline oxidase synthesis in *Klebsiella aerogenes* (16). Magasanik et al. (13) have pointed out that certain amino acids such as histidine and proline are capable of producing glutamate through the action of histidase and proline oxidase. The synthesis of these enzymes is subject to CR; however, they are synthesized in the presence of glucose if histidine or proline must serve as the sole source of glutamate. It may be that because DS does not give rise to glutamate, Dsdase synthesis need not be responsive to the supply of nitrogen in the medium, or it may be that the *K. aerogenes* nitrogen starvation escape mechanism does not exist in *E. coli*.

ACKNOWLEDGMENTS

We thank Louise Huebsch for help with the chemostat experiments and media.

This work was supported by Public Health Service Research Grant no. 11899; F. R. B. was supported by Training Grant No. 01290; an E. M. was a Career Development Awardee, Grant no. GM7390, all from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitroso-guanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788-795.
2. Bloom, F. B., E. McFall, M. C. Young, and A. M. Carothers. 1975. Positive control of the D-serine deaminase system of *Escherichia coli* K-12. J. Bacteriol. **121**:1092-1101.
3. Cosloy, S. D., and E. McFall. 1973. Metabolism of D-serine in *Escherichia coli* K-12: mechanism of growth inhibition. J. Bacteriol. **114**:685-694.
4. Dupourque, D., W. A. Newton, and E. E. Snell. 1966. Purification and properties of D-serine dehydrase from *Escherichia coli*. J. Biol. Chem. **241**:1233-1237.
5. Englesberg, E., J. Irr, J. Power, and N. Lee. 1965. Positive control of enzyme synthesis by gene C in the L-arabinose system. J. Bacteriol. **90**:946-957.
6. Luria, S. E., J. N. Adams, and R. C. Ting. 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology **12**:348-390.
7. McFall, E. 1964. Genetic structure of the D-serine deaminase system of *Escherichia coli*. J. Mol. Biol. **9**:746-753.
8. McFall, E. 1964. Pleiotropic mutations in the D-serine deaminase system of *Escherichia coli*. J. Mol. Biol. **9**:754-762.
9. McFall, E. 1967. Mapping of the D-serine deaminase region in *Escherichia coli* K12. Genetics **55**:91-99.
10. McFall, E. 1967. Dominance studies with stable merodiploids in the D-serine deaminase system of *Escherichia coli* K-12. J. Bacteriol. **94**:1982-1988.
11. McFall, E. 1973. Role of adenosine 3'-5'-cyclic monophosphate and its specific binding protein in the regulation of D-serine deaminase synthesis. J. Bacteriol. **113**:781-785.
12. McFall, E. 1975. *Escherichia coli* K-12 mutant forming a temperature-sensitive D-serine deaminase. J. Bacteriol. **121**:1074-1077.
13. Magasanik, B., M. J. Prival, J. E. Brenchley, B. M. Tyler, A. B. DeLeo, S. S. Streicher, R. A. Bender, and C. G. Paris. 1974. Glutamine synthetase as a regulator of enzyme synthesis, p. 119-138. *In* B. Horecker and E. Stadtman (ed.), Current topics in cellular regulation. Academic Press Inc., New York.
14. Mandelstam, J. 1962. The repression of constitutive β-galactosidase in *Escherichia coli* by glucose and other carbon sources. Biochem. J. **82**:489-493.
15. Novick, A., and L. Szilard. 1960. Experiments with the chemostat on spontaneous mutations of bacteria. Proc. Nat. Acad. Sci. U.S.A. **36**:708-719.
16. Prival, M. J., and B. Magasanik. 1971. Resistance to catabolite repression of histidase and proline oxidase during nitrogen-limited growth of *Klebsiella aerogenes*. J. Biol. Chem. **246**:6288-6296.
17. Stacey, K. A., and E. Simson. 1965. Improved method for the isolation of thymine-requiring mutants of *Escherichia coli*. J. Bacteriol. **90**:554-555.
18. Taylor, A. L., and C. D. Trotter. 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36**:505-524.