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Effects of Dimethylsulfoxide on the Lactose Operon in *Escherichia coli*

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ABSTRACT

FOWLER, AUDREE V. (University of California, Los Angeles), AND IRVING ZABIN. Effects of dimethylsulfoxide on the lactose operon in *Escherichia coli*. J. Bacteriol. **92**:353–357. 1966.—Dimethylsulfoxide (DMSO) at a concentration of 5% (v/v) in the culture medium inhibits the growth of *Escherichia coli* to only a slight extent, and does not affect the differential rate of synthesis of β-galactosidase. Resting cells remain viable after shaking in the presence of 20% DMSO for 3 hr at 37 C. Both β-galactosidase and thiogalactoside transacetylase retain almost all activity after incubation in even higher concentrations of the solvent for many hours. DMSO decreases the permeability barrier. The rate of hydrolysis of *o*-nitrophenyl-β-D-galactoside (ONPG) in whole cells containing β-galactosidase but lacking permease is increased in cells treated with 5% DMSO. Several permeaseless strains preinduced for β-galactosidase will grow on lactose in the presence, but not in the absence, of 5% DMSO. When permeaseless strains are grown on tetrazolium-lactose-agar, the presence of 5% DMSO causes a definite but not marked shift toward the lactose-positive character.

The lactose operon in *Escherichia coli* contains structural genes for β-galactosidase, galactoside permease, and thiogalactoside transacetylase. Work in this laboratory has been concerned with the structure and the rates of synthesis of the two well-defined enzymes, β-galactosidase and thiogalactoside transacetylase, as well as with a possible physiological role for the latter.

In the course of these studies, attempts were made to alter the permeability of *E. coli* to various materials. Dimethylsulfoxide (DMSO) was chosen as a reagent for this purpose for several reasons. It has excellent solvent properties and is miscible in all proportions with water and with many organic solvents. Many biological macromolecules such as proteins (2, 9), nucleic acids (3), and carbohydrates are not only soluble in anhydrous DMSO, but remain active. For example, trypsin is active in 95% DMSO (2). Mammalian cells have been preserved at low temperatures in the presence of DMSO (1, 8), and studies have been carried out on the penetrating ability of this solvent.

We have investigated the effects of DMSO on growth rate, on induction of β-galactosidase, on the activities of the purified enzymes, and on permeability.

MATERIALS AND METHODS

Coenzyme A and isopropyl-β-D-thiogalactoside (IPTG) were purchased from Calbiochem. Anhydrous DMSO (Spectroquality Reagent Grade) was obtained from Matheson, Coleman and Bell, East Rutherford, N.J. All other reagents used were obtained as indicated previously (10).

The following strains were obtained from J. Monod, Pasteur Institute: ML 30 ($i^+z^+y^+$); ML 3 ($i^+z^+y^-$); ML 308 ($i^-z^+y^+$); K-12 3300 ($i^-z^+y^+$); and K-12 300 U ($i^+z^+y^-$). A. Rich, Massachusetts Institute of Technology, kindly provided Hfr 3000 ($i^+z^+y^+$); 707 y^- amber ($i^+z^+y^-$); and W 6203 ($i^+z^+y^-$). The symbols *i*, *z*, and *y* are the usual notations for the regulatory gene, the β-galactosidase gene, and the galactoside-permease gene, respectively.

Mineral salts medium 63 (4) was used in all experiments. Thiamine (1 μg/ml) was added to the culture medium of all strains except ML.

Thiogalactoside transacetylase was purified and assayed as described previously (10). β-Galactosidase was isolated (6) and was measured by the procedure of Horiuchi et al. (5) with the use of the PM 2 buffer containing 0.05 M mercaptoethanol. Growth was followed by turbidity at 600 mμ.

Samples of overnight cultures were used to inoculate fresh medium. At midlog phase, the cells were harvested by centrifugation, washed with medium 63, and suspended in the media indicated. Incubations

were carried out in a rotating water bath at 37 C or on a reciprocal shaker in a room at 37 C.

Total β-galactosidase levels in cells were measured after shaking 1-ml samples of cultures with 1 drop of toluene and 1 drop of 1% sodium deoxycholate at 37 C for 1 hr.

The concentrations of DMSO used were per cent by volume. Culture media contained the same amount of salt in all cases.

RESULTS

Effect of DMSO on growing cells. A typical effect of the presence of DMSO in the culture medium on the growth of a wild-type strain, ML 30, with succinate as a carbon source, is shown in Fig. 1. At concentrations below 5%, no effect on growth rate was observed, and even at 5%, the doubling time of the cells was reduced to a small extent. Marked inhibition occurred at 10% DMSO, and concentrations of 20% and higher completely inhibited growth.

In general, the same effect of DMSO was found with ML and K-12 strains, and with other carbon sources such as glycerol or lactose. With 5% DMSO, the change in doubling times varied from culture to culture, but an increase of more than 25 to 50% was rarely observed.

Effect of DMSO on induction of β-galactosidase. The wild-type strain ML 30 was grown in different concentrations of DMSO in the presence and absence of inducer, and growth and β-galactosidase formation were measured. The data in Table 1 show that the growth rate and β-galactosidase formation were inhibited to the same extent, indicating that the differential rate of synthesis of the enzyme remained the same.

Effect of DMSO on resting cells. Bacteria may be shaken in the presence of relatively high concentrations of DMSO in mineral medium containing no carbon source without affecting subsequent growth in DMSO-free medium. In the experiment illustrated in Fig. 2, a culture of ML 30 in the exponential phase of growth on succinate was centrifuged and washed, and the cells were suspended in medium 63 containing varying concentrations of DMSO. After shaking for 3 hr at 37 C, the cells were harvested, washed, and resuspended in medium 63 and succinate. Essentially no effect on growth rates was detected after preincubation in concentrations of DMSO up to 20%. Preincubation with 40% DMSO, however, prevented all growth. Similar results were obtained with a galactoside-permeaseless strain, 707.

Viability after DMSO treatment was determined with another y^- strain, ML 3. After growth on succinate in the presence of 1.5×10^{-3} M inducer, a culture was harvested and washed, and the cells were shaken at 37 C in medium 63 in the presence and absence of 5% DMSO for 1, 4, and 8 hr. By plating and direct colony counting, it was found that no loss in viability occurred due to the presence of DMSO, even after 8 hr. Only trace amounts of β-galactosidase were found in the supernatant solutions after these shaking periods, whether or not DMSO was present.

Leakage of ultraviolet-absorbing material into the medium as a consequence of the presence of DMSO was also examined. Cultures of ML 30 were shaken for 3 hr at 37 C at a concentration

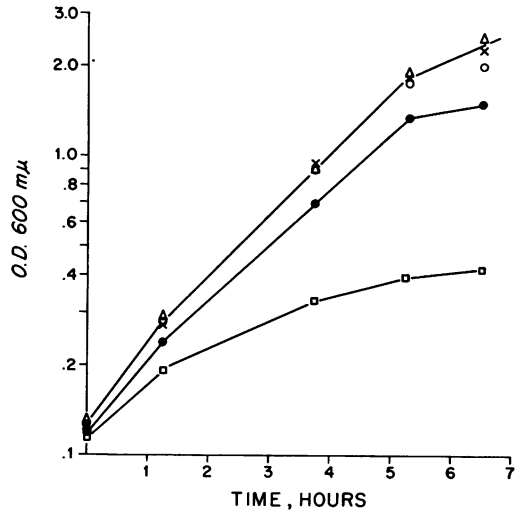

FIG. 1. *Growth of ML 30 in the presence of DMSO. Cells growing in the exponential phase on 0.4% succinate were inoculated into fresh medium containing 0.4% succinate and varying amounts of DMSO. Symbols:* ×, *control;* ○, *0.5%;* △, *1%;* ●, *5%; and* □, *10% DMSO.*

TABLE 1. *Effect of DMSO on induction of β-galactosidase*[a]

DMSO	Inhibition of growth		Inhibition of β-galactosidase Formation
	Noninduced	Induced	
%	%	%	%
0	—	—	—
5	48	59	54
10	94	87	91
20	100	100	100

[a] A culture of ML 30 in the exponential phase of growth on succinate was transferred to fresh medium containing DMSO and 5×10^{-4} M IPTG as indicated. After 4.5 hr of growth, the cells were still in the exponential phase. Turbidity was measured; the cells were centrifuged, treated with toluene, and assayed for β-galactosidase.

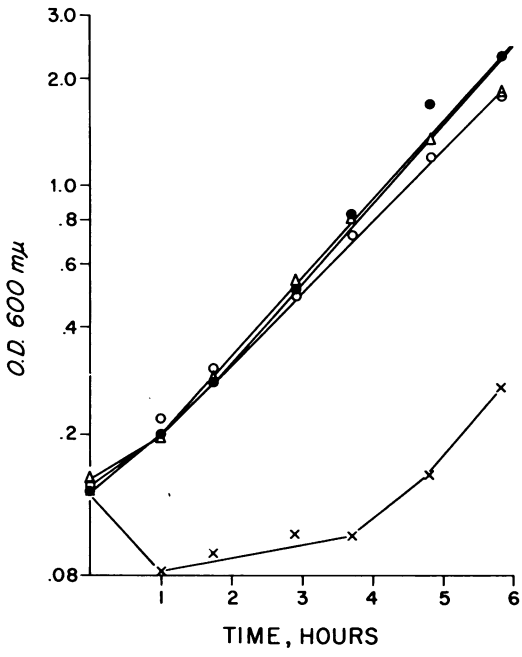

FIG. 2. *Effect of treatment of resting cells with DMSO on subsequent growth. Cultures of ML 30 grown to the exponential phase on 0.4% succinate were centrifuged, washed with succinate-free medium, and resuspended at 4 × 10⁷ cells per milliliter in succinate-free medium containing: ○, 0%; ●, 10%; △, 20%; and ×, 40% DMSO. After shaking for 3 hr at 37 C, the cells were centrifuged, washed, and resuspended in mineral medium containing 0.4% succinate. Growth was followed by turbidity.*

of 10 mg (dry weight) of bacteria in medium 63 in the presence and absence of 10% DMSO. No greater quantity of material absorbing at 260 mμ was found in the supernatant solution from the suspension containing the organic solvent as compared with the solvent-free suspension.

Effect of DMSO on β-galactosidase. The enzyme remains quite stable when allowed to stand in the presence of DMSO for relatively long periods of time. At a concentration of 2 mg/ml, 80% of the enzymatic activity was retained after 20 hr in 20% DMSO (Table 2). In 40% DMSO, however, almost all activity was lost in the same length of time, although activity is retained for 1 hr in 40% DMSO. Lower concentrations of enzyme (0.02 mg/ml) result in decreased stability in DMSO.

The analyses shown in Table 2 were carried out by diluting the enzyme into the assay mixture so that insignificant amounts of DMSO remained. When the assay was carried out in the presence of 10 and 20% DMSO, inhibitions of activity of 15 and 30%, respectively, were observed.

β-Galactosidase inactivated with 50% DMSO could not be reactivated by dialysis against 0.01 M tris(hydroxymethyl)aminomethane (pH 7.7), 2×10^{-4} M mercaptoethanol, and 5×10^{-4} M manganese sulfate.

Effect of DMSO on thiogalactoside transacetylase. The stability to incubation with DMSO of this enzyme is even greater than that of β-galactosidase (Table 3). Essentially no loss of activity was observed after 5 hr in 40% DMSO.

Effect of DMSO on permeability to o-nitrophenyl-β-D-galactoside (ONPG). Cells were shaken in medium 63 containing 5 and 10% DMSO, and were centrifuged, washed, and resuspended in medium 63. One portion was tested for β-galactosidase activity with ONPG after breaking the cells by toluenization; the activity in

TABLE 2. *Effect of DMSO on β-galactosidase*[a]

Protein	Time	DMSO				
		0%	10%	20%	40%	50%
mg/ml	*hr*					
2.0	0.25	102[b]	93	86	100	
	1.0	95	100	94	84	
	4	92	89	88	36	
	20	93	87	80	1.5	
0.02	4		100			7
	20		9			2

[a] β-Galactosidase in PM-2 buffer (*p*H 7.0) containing 0.05 M mercaptoethanol was incubated at 37 C as indicated, and then was diluted 100-fold into DMSO-free buffer before activity measurement.

[b] Percentage of initial activity remaining.

TABLE 3. *Effect of DMSO on thiogalactoside transacetylase*[a]

Time	DMSO					
	0%	4%	10%	20%	40%	50%
hr						
0.25	115[b]			104	112	
1.0		93	105	113	86	
5	109	90	95	99	94	20
24	105	77	76	63	16	13

[a] A preparation of enzyme 50% pure was incubated at 37 C at a protein concentration of 3.24 mg/ml in 0.1 M potassium phosphate buffer (*p*H 7.2) containing 5×10^{-3} M mercaptoethanol. At the times indicated, the enzyme was diluted 100-fold into DMSO-free buffer before activity measurement.

[b] Percentage of initial activity remaining.

another sample was measured with whole cells. The crypticities, i.e., the ratio of the rates of hydrolysis in broken versus unbroken cells, are shown in Table 4. When permeaseless strains were tested, a clear decrease in the permeability barrier was found, with decreases in crypticity up to 50%. Permease-positive strains also showed this effect, but results were more variable.

Growth on lactose of permeaseless strains with DMSO. Because permeability to ONPG was increased by DMSO, it seemed possible that strains which could not grow on lactose because galactoside-permease is absent might be able to do so in the presence of DMSO. This proved to be the case (Fig. 3). When a culture of ML 3 which was previously induced with IPTG while growing on succinate was harvested, washed, and transferred to medium containing lactose and 5% DMSO, the cells grew at the same rate, after a lag period, as a culture growing on succinate containing 5% DMSO. The lag period may be ascribed to the time required to increase permeability to lactose by DMSO. It can be seen that, in the absence of the solvent, little growth occurred on lactose.

Similar results were obtained with K-12 300 U. Several other permeaseless strains preinduced for β-galactosidase were found to grow on lactose in the absence of DMSO. No permeaseless strain, however, grew on lactose whether or not DMSO was present unless previously induced with IPTG.

Growth of permeaseless strains on agar containing DMSO. On Penassay Base Agar plates containing tetrazolium and lactose, y^+ cells are white and y^- cells are bright red (7). Depending on the strain, intermediate shades including a dull red or pink are also observed. With 5% DMSO incorporated into the agar, a shift toward white was observed with several permeaseless strains; this phenomenon was enhanced when IPTG was also present. These changes in color, while obvious when a plate containing DMSO was compared with one without the solvent, were not great, suggesting that the use of DMSO in selection methods would be difficult. The growth rate of the cells as judged by colony size did not appear to be affected by 5% DMSO.

When the solvent was incorporated into MacConkey Agar, the color shifted from red toward yellow, making the selection of lactose-positive colonies difficult. No further work with this indicator was carried out.

TABLE 4. *Effect of DMSO on permeability*

Strain	Time	Crypticity factor		
		0% DMSO	5% DMSO	10% DMSO
	hr			
Permease present				
ML 30 (induced)	2	17	13	10
ML 30 (induced)	2	19	19	11
Permease absent				
ML 3	1	189	149	
	4	145	90	
	8	152	67	
ML 3	2	162		83
707	2	153		106
6203	2	148		109

[a] Cultures were shaken at 37 C in medium 63 containing DMSO as indicated; the cells were centrifuged and resuspended in medium 63. The crypticity factor is the rate of ONPG hydrolysis in toluene-treated cells compared with that in whole cells.

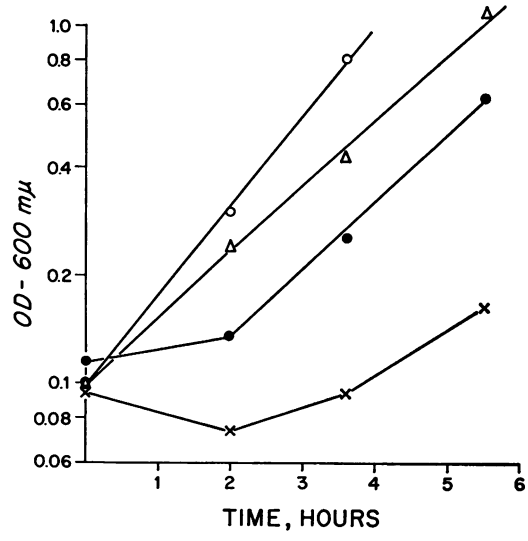

FIG. 3. *Growth of a permeaseless strain on lactose in the presence of DMSO. A culture of ML 3 was grown to midexponential phase in medium containing 0.4% succinate and 1.5 × 10⁻³ M IPTG. The cells were centrifuged, washed, and resuspended in medium containing: ○, 0.4% succinate; △, 0.4% succinate and 5% DMSO; ●, 0.4% lactose and 5% DMSO; and ×, 0.4% lactose.*

DISCUSSION

The most interesting observation obtained in these studies is that DMSO can alter permeability of *E. coli*. Pretreatment of cells in mineral medium with concentrations of 10% solvent caused a decrease in the crypticity factor in strains lacking galactoside-permease. Furthermore, several of these strains which do not grow on lactose were found to grow on this sugar in the presence of 5% DMSO, provided that β-galactosidase had been previously induced. It is possible that these results might be due to a selective effect of DMSO

on a small proportion of cells, causing an apparent rather than a real decrease in crypticity of each cell. Growth on lactose might be due to destruction of some cells in the population with consequent exposure of lactose to β-galactosidase, and growth of unaffected cells on the glucose and galactose produced by hydrolysis. We believe that such a selective effect is unlikely, and that the decrease in permeability is a real effect on most cells in these cultures. No loss in viability was observed even after shaking cultures with relatively high concentrations of DMSO, no excess of ultraviolet-absorbing material was released into the medium, and no β-galactosidase could be detected in more than trace amounts in supernatant solutions after DMSO treatment.

These results, then, suggest that DMSO may be a useful reagent for studies in which entry of sugar molecules and possibly other small molecules into bacterial cells is desired. Because the low concentrations of DMSO which are effective in reducing the permeability barrier to ONPG and lactose have no effect on activity of the two enzymes tested, β-galactosidase and thiogalactoside transacetylase, it is probable that irreversible effects on other components of the cell do not occur. It would appear that DMSO can be used for a variety of studies with bacteria as well as with mammalian cells.

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LITERATURE CITED

1. ASHWOOD-SMITH, M. J. 1961. Preservation of mouse bone marrow at −79°C. with dimethyl sulfoxide. Nature **183**:1394–1395.
2. BETTLEHEIM, F. A., K. SMITH, AND A. LUKTON. 1962. Trypsin can function in anhydrous medium. Chem. Eng. News **40**(20):41–42.
3. HELMKAMP, G. K., AND P. O. P. TS'O. 1961. The secondary structure of nucleic acids in organic solvents. J. Am. Chem. Soc. **83**:138–142.
4. HERZENBERG, L. A. 1959. Studies on the induction of β-galactosidase in a cryptic strain of *Escherichia coli*. Biochim. Biophys. Acta **31**:525–539.
5. HORIUCHI, T., J. TOMIZAWA, AND A. NOVICK. 1962. Isolation and properties of bacteria capable of high rates of β-galactosidase synthesis. Biochim. Biophys. Acta **55**:152–163.
6. KARLSSON, U., S. KOORAJIAN, I. ZABIN, F. S. SJOSTRAND, AND A. MILLER. 1964. High resolution electron microscopy on highly purified β-galactosidase from *Escherichia coli*. J. Ultrastruct. Res. **10**:457–469.
7. LEDERBERG, J. 1950. Isolation and characterization of biochemical mutants of bacteria. Methods Med. Res. **3**:5–22.
8. LOVELOCK, J. E., AND M. W. H. BISHOP. 1959. Prevention of freezing damage to living cells by dimethyl sulfoxide. Nature **183**:1394–1395.
9. REES, E. D., AND S. J. SINGER. 1956. A preliminary study of the properties of proteins in some nonaqueous solvents. Arch. Biochem. Biophys. **63**:144–159.
10. ZABIN, I. 1963. Crystalline thiogalactoside transacetylase. J. Biol. Chem. **238**:3300–3306.