THE EFFECT OF DIMETHYL SULFOXIDE (DMSO) ON PERMEABILITY OF STAPHYLOCOCCUS AUREUS

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Received August 1, 1968

In recent years a great deal of attention has been devoted to finding biological and therapeutic uses of dimethyl sulfoxide. Many studies have been made on the reduction of permeability barriers of plant and animal cells when this compound was used in conjunction with chemicals and drugs (see Vol. 141 (1967), Ann. N. Y. Acad. Sci.). Only limited studies concerning the effect of DMSO on microorganisms have been made, and very little is known about the mode of action of DMSO. This report concerns some studies made on the effect of DMSO on permeability of Staphylococcus aureus. Treating staphylococci with DMSO was found to result in a greater rate of oxygen and lactose uptake, while the rate of glycine transport was reduced. Some possible mechanisms for the mode of action of this compound are also discussed.

MATERIALS AND METHODS

The cultures employed in this study were S. aureus strain NCTC 8511 (also designated as strain 53) and a mutant of this strain which was obtained from Dr. M. L. Morse of the University of Colorado Medical School. The mutant had lost the ability to utilize lactose and thus was designated as lac$^-$ (Egan and Morse, 1965). Dimethyl sulfoxide was obtained from Sigma Chemical Company and a concentration of 5% was routinely used. The incubation period with DMSO was three hours prior to the addition of the radioactive substrate.

For oxygen uptake studies, the optical densities of washed cells were adjusted to give an absorbancy of 0.7 at 600 mμ. The rate of O_2 uptake was measured by a Gilson oxygraph model KM. The dry weights of the samples were determined by collecting a one ml sample on HA Millipore filters. The amount of protein in the sample was determined by the method of Lowry et al (1951) after hydrolysis with 1 M NaOH, using bovine serum albumin as the standard.

Cells were prepared as described above for lactose and glycine studies. They were incubated for 30 minutes with 25 μg/ml of chloramphenicol to stop protein synthesis. Final concentrations of lactose-1-C^{14} and glycine-2-C^{14} were 2.5×10^{-5} and 3.7×10^{-5} respectively. Samples were collected on HA millipore filters, washed, counted in a planchet counter (Nuclear Chicago model 181A), and weighed.

RESULTS

The data in Table 1 indicate that lac$^-$ cells utilized oxygen at a lower rate than the parent cells. The addition of DMSO appeared to increase the rate of O_2 uptake in strain 53 and lac$^-$ cells. This increase in oxygen consumption was to the same extent in both strains.

Table 1. Effect of DMSO on oxygen utilization by S. aureus strains 53 and lac$^-$.

Strain	O_2 Uptake μ moles/sec/mg protein	
	Without DMSO	With DMSO
53	0.304	0.320
lac$^-$	0.212	0.224

When DMSO was added to cells of strain 53 an increase in the rate of lactose uptake was observed in comparison with the untreated cells (Figure 1). In contrast, addition of DMSO to lac$^-$ cells did not result

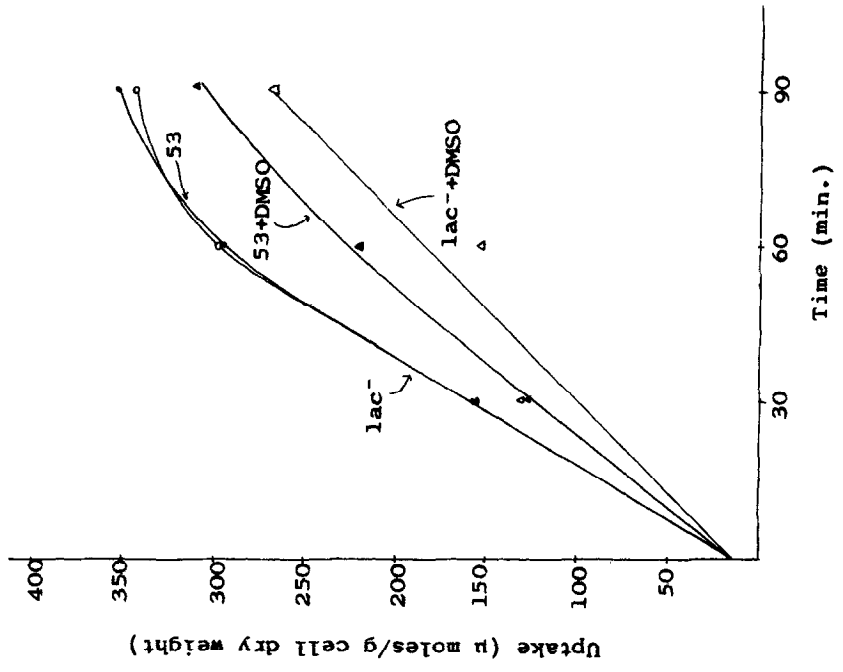

FIGURE 2. The comparative uptake of glycine by S. aureus strains 53 and lac⁻ in the presence and absence of DMSO.

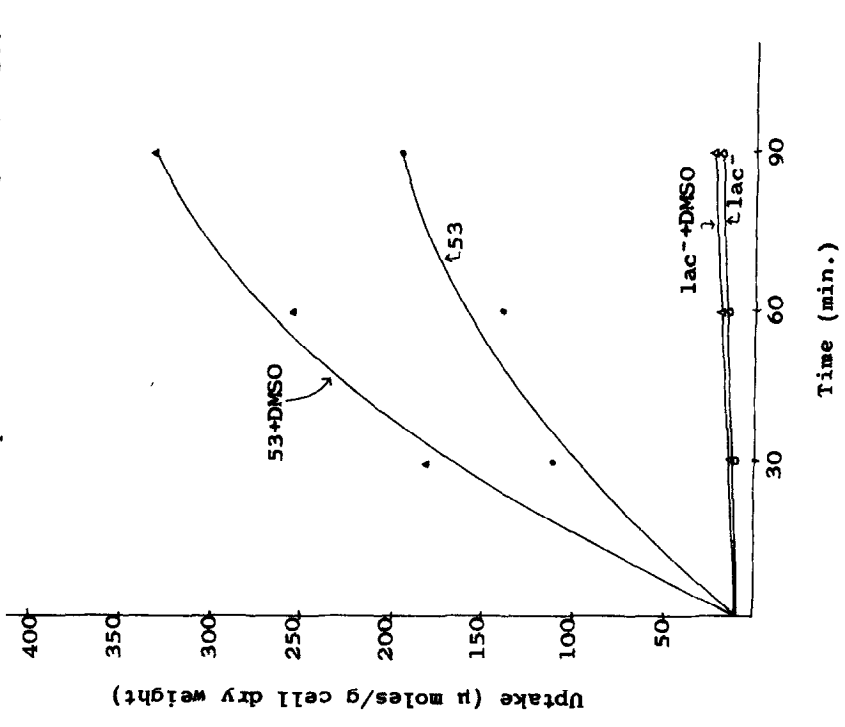

FIGURE 1. The comparative uptake of lactose by S. aureus strains 53 and lac⁻ in the presence and absence of DMSO.

in a significant difference in the rate of lactose uptake.

The results of amino acid uptake studies indicated that lac$^-$ cells were capable of taking up glycine with a rate comparable to the parent strain 53. DMSO, however, seemed to hinder the transport of glycine, and the inhibition was to the same extent in both strains (Figure 2).

Experiments were also done in which the cells were incubated with DMSO, but washed free of this agent before addition of glycine. The results indicated that removal of DMSO from the medium restored the normal glycine transport.

DISCUSSION

In addition to simple diffusion another permeation system for carbohydrate transport in Escherichia coli was discovered by Cohen and Monod (1957). Later Kepes (1960) suggested a model for this transport system which contained specific permeases and a common carrier necessary for transport of sugars. Some proteins which are associated with the permease system of E. coli have been isolated by Fox and Kennedy (1965), and a phosphotransferase system has been described by Kundig et al (1964).

A pleiotropic mutant of S. aureus (designated car$^-$) was isolated by Egan and Morse (1965) which could not utilize a number of carbohydrates. The genetic and biochemical analyses of car$^-$ cells led these investigators (Hengstenberg et al, 1967) to conclude that the car mutation was the result of a defect in a phosphotransferase system which is similar to that suggested for E. coli.

In an investigation of the effect of DMSO on the lactose operon of E. coli, β-galactosidase was found to be stable in low concentrations of DMSO (Fowler and Zabin, 1966). The investigation of Rammler (1967) showed that the activities of some enzymes increase in the presence of DMSO, while there is a reduction in the activity of most enzymes tested in vitro. It was felt that this change in enzyme

activity was due to the removal of bound water from protein molecules by DMSO.

The data presented here indicated that the presence of DMSO increased the oxygen uptake, but reduced the rate of glycine transport in both strains. This agent also increased the rate of lactose uptake in strain 53 but had no apparent effect on lac$^-$ cells. Although it is difficult to state the exact mechanism by which DMSO acts on the bacterial membrane at this time, certain effects of this compound are clear. First, one effect may be on membrane proteins causing reversible alterations in their structure. Secondly, DMSO is an excellent fat solvent and it has been shown to remove some fatty acids from the bacterial membrane (Adams, 1967). Thus it is possible that facilitated transport of certain compounds may occur as a result of porosity of the membrane. Thirdly, the enhancement in the rate of oxygen uptake suggests a surface activation behavior, perhaps caused by mediation of DMSO between the polar and non-polar phases.

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