ROLE OF GLUTAMINE SYNTHETASE IN THE REPRESSION OF BACTERIAL SPORULATION.

Claudine Elmerich and Jean-Paul Aubert
Service de Physiologie Cellulaire, Institut Pasteur, Paris.

Received December 6, 1971

In a glucose ammonia minimal medium not supplemented with uracil, the uracil negative strain MA22 of B.megaterium cannot grow and its sporulation remains repressed. With the aid of mutants deficient either in glutamine synthetase or in glutamine (amide) : 2-oxo-glutarate aminotransferase (NADP oxidoreductase) it is shown that the first enzyme involved in the repression of sporulation is the glutamine synthetase and that the organic nitrogen containing compound required for repression is glutamine but not glutamate.

Earlier studies (1, 2) concerning the pathway of glutamate synthesis in B.megaterium have shown that glutamate is synthesized by the coupled functioning of a glutamine synthetase (GlnS) and of a glutamine (amide) : 2-oxo-glutarate aminotransferase (NADP oxidoreductase) termed here glutamate synthase (GluS). Because B.megaterium has neither an aspartase nor a glutamate dehydrogenase and because alanine dehydrogenase is not involved in alanine synthesis (unpublished results), GlnS would appear to be the major enzyme involved in ammonia incorporation.

This raises the question of whether GlnS plays a role in the repression of sporulation. According to Schaeffer's et al. hypothesis, sporulation would be repressed by nitrogen-containing catabolites (3). In that case, glucose and ammonia might be considered as the first substrates in a sequence of

reactions the last of which acts to repress sporulation. As will be shown, GlnS is the first in the series of enzymes that are involved in the reaction chain. Subsequently repression of sporulation is derived from glutamine but not from glutamate.

MATERIAL AND METHODS

The experimental procedure for studying sporulation had first been developed with B.megaterium MA22, a mutant that requires uracil for growth but not for sporulation (4). Strain MA22 was grown in complex medium (5) until just before the end of growth when it was transferred to one of four sporulation media, containing the salt solution of the complex medium plus either 2 mg glucose/ml (G) and/or 0.038 M ammonium chloride (N). All media were free of uracil. Good sporulation occured in the G-N-, G-N+ and G+N- media, but sporulation was repressed in the G+N+ medium. These results are in agreement with the Schaeffer's et al hypothesis (3).

Isolation of mutant $Gln^{-}26$, devoid of GlnS activity, and of mutant $Glu^{-}11$, devoid of GluS activity has been described previously (2). Revertants $Gln^{-}26$ R 1 and $Glu^{-}11$ R 1 were isolated by their capacity to grow on glucose ammonia minimal agar. All strains sporulated normally after growth in complex medium.

RESULTS

Sporulation of MA22, $Gln^{-}26$, $Gln^{-}26$ R 1, $Glu^{-}11$ and $Glu^{-}11$ R 1 was studied in the four transfer media. Results reported in Table I show that : 1) sporulation of the two revertants was similar to that of MA22 ; 2) both $Gln^{-}26$ and $Glu^{-}11$ differed from MA22 and from each other in that sporulation of $Gln^{-}26$, in the G+N+ medium, was less repressed than that of MA22 whereas sporulation of $Glu^{-}11$ was more repressed than that of MA22 in the same medium.

Due to the need for the combined action of GlnS and

Table I. Sporulation of B.megaterium MA22 strain, of Gln⁻26 and Glu⁻11 mutants and of Gln⁻26 R 1 and Glu⁻11 R 1 revertants.

	Transfer media			
	G-N-	G-N+	G+N-	G+N+
MA22	44	45	30	0.2
Gln⁻26	50	50	7	8
Glu⁻11	6	3	< 0.001	< 0.001
Gln⁻26 R 1	45	26	22	0.7
Glu⁻11 R 1	40	45	28	0.2

Sporulation is expressed as %. Each media contained about 25 x 10^6 cells/ml.

GluS in glutamate synthesis, neither Gln⁻26 nor Glu⁻11 can synthesize glutamate. Since, in Bacilli, glutamate accumulates inside the sporangium in the early stages of sporulation (5, 6, 7) and serves as a precursor of many other metabolites (5), it was necessary to study how glutamate addition, at a growth factor concentration, affected sporulation particularly in the G+N- and G+N+ media.

From the results reported in Table II it can be seen that, in the case of Gln⁻26 the addition of 20 µg glutamate/ml led to normal sporulation in both media. However glutamate addition had little effect on sporulation of MA22 or of Glu⁻11.

The fact that supplementation of G+N+ medium with 20 µg glutamate/ml allowed Gln⁻26 to sporulate suggests that the GlnS⁻ mutation permits the strain to overcome the repression of sporulation by nitrogen-containing catabolites. If similar reasoning is applied to the behavior of Glu⁻11, the opposite conclusion is arrived at for the mutation GluS⁻. In other words GlnS appears to participate in the repression of sporulation and GluS would not appear to do so.

If this conclusion is correct, the first product of GlnS activity, i.e. glutamine, should repress sporulation of

TABLE II. Influence of glutamate on the sporulation of B.megaterium MA22 strain and of Gln⁻26 and Glu⁻11 mutants.

	Transfer media			
	G+N-		G+N+	
	-Glu	+Glu (20 µg/ml)	-Glu	+Glu (20 µg/ml)
MA22	30	57	0.2	0.2
Gln⁻26	7	62	8	70
Glu⁻11	<0.001	0.05	<0.001	<0.001

Sporulation is expressed as %. Each medium contained about 25 x 10^6 cells/ml.

Table III. Effect of increasing concentrations of glutamine and glutamate on the sporulation of Gln⁻26 B.megaterium mutant in the G+N+ medium.

Concentrations (µg/ml)	+Gln	+Glu
0	7	7
20	60	62
50	40	56
100	16	60
200	2	66

Sporulation is expressed as %. Each media contained about 25 x 10^6 cells/ml.

Gln⁻26 and the first product of GluS activity, i.e. glutamate, should not. Results reported in Table III show that this was indeed the case when increasing concentrations of glutamine and glutamate were added to the G+N+ medium. The activating effect of glutamine at low concentration is readily explained by the

fact that Gln$^-$26 is GluS$^+$ and therefore can rapidly transform glutamine to glutamate.

DISCUSSION

In B.megaterium, GlnS is the major enzyme responsible for ammonia incorporation. As sporulation of Gln$^-$26 is insensitive to repression by nitrogen-containing catabolites when the medium contains both glucose and ammonia, it may be concluded that GlnS is the initial enzyme involved in sporulation control.

On the other hand two kinds of results lead to the conclusion that repression of sporulation depends on glutamine and not on the closely related glutamate : 1) sporulation of Gln$^-$26 in the G+N+ medium is insensitive to the addition of high concentrations of glutamate, but is repressed by the addition of high concentrations of glutamine ; 2) sporulation of Glu$^-$11, which is GluS$^-$ and GlnS$^+$, is most highly repressed in the G+N+ medium.

One point that deserves discussion is whether glutamine acts through a specific or through a non-specific pathway. Glutamine can be metabolized via the Krebs cycle but only after conversion to glutamate. The fact that glutamine and not glutamate represses sporulation of Gln$^-$26 rules out the possibility that this repression resulted from glutamine having been metabolized via the Krebs cycle. One may therefore conclude that repression of sporulation is related either to glutamine itself or to another compound derived from glutamine, but not from glutamate.

ACKNOWLEDGMENTS

We thank Mrs. Mireille Poulin for skilful technical assistance. This investigation was supported by grant 70-02-264 from the Délégation Générale à la Recherche Scientifique et Technique.

REFERENCES

1. J.L. Meers, D.W. Tempest, and C.M. Brown, J.Gen.Microbiol., 64, 187 (1970).

2. C. Elmerich and J.P. Aubert, Biochem.Biophys.Res.Comm., 42, 371 (1971).
3. P. Schaeffer, J. Millet, and J.P. Aubert, Proc.Nat.Acad.Sci., 54, 704 (1965).
4. C. Elmerich, C.R.Acad.Sc., 271, 134 (1970).
5. J. Millet and J.P. Aubert, Ann.Inst.Pasteur, 98, 282 (1960).
6. H.H. Martin and J.W. Foster, J.Bact., 76, 167 (1958).
7. R.W. Bernlohr, J.Bact., 93, 1031 (1967).