SHORT COMMUNICATION

Selective Inactivation of Nitrogenase and Glutamate Synthase during Sporulation of *Clostridium pasteurianum*

By F. VALLESPINÓS† AND D. KLEINER*

Chemisches Laboratorium der Universität, Albertstrasse 21, D-7800 Freiburg, German Federal Republic

(Received 12 October 1979; revised 3 December 1979)

When sporulation was induced in batch cultures of N_2 -fixing *Clostridium pasteurianum*, either by adding calcium acetate or by increasing the pH, inactivation of glutamate synthase, partial inactivation of nitrogenase and excretion of NH_4^+ were observed. Glutamine synthetase activity remained unchanged during sporulation.

INTRODUCTION

The formation of bacterial spores or cysts is characteristically accompanied by extensive protein turnover and inactivation of numerous enzymes. Induction of encystment in *Azotobacter vinelandii* was found to cause rapid inactivation of nitrogenase, while the other enzymes of the primary steps of N_2 and NH_4^+ assimilation – glutamine synthetase (EC 6.3.1.2) and glutamate synthase (EC 1.4.1.13) – remained fully active for at least 3 h (Hitchins & Sadoff, 1973; Kleiner & Kleinschmidt, 1976). Depending on the method used to induce encystment, variable amounts of NH_4^+ were excreted.

As far as we know, no similar studies have been undertaken with other cyst- or spore-forming organisms capable of fixing N_2 . In the present paper we report some investigations which demonstrate rapid inactivation of nitrogenase and glutamate synthase during sporulation of *Clostridium pasteurianum*.

METHODS

Clostridium pasteurianum (a gift from Professor R. H. Burris) was grown at 30 °C in batch cultures with automatic pH control (pH 6.0) in the medium described by Kleiner (1979) supplemented with 75 mg $CaCl_2$ ml^{-1} . The anaerobic preparation of crude extracts, and the determination of NH_4^+ , nitrogenase activity both *in vivo* and *in vitro*, glutamine synthetase (Mg^{2+} -dependent synthetase activity) and glutamate synthase were carried out as outlined by Kleiner (1979).

Sporulation was induced either by adding calcium acetate to a final concentration of 0.1 M, or by increasing the pH from 6.0 to 8.4 by adding concentrated KOH. When cultures with an A_{660} between 1.2 and 1.6 were treated in this way, both methods led to almost 100% spore formation within 2 h and gave essentially the same results with respect to the changes in enzyme activities. Since the reproducibility was higher with the second method, only these results are reported.

RESULTS AND DISCUSSION

Spore formation in *C. pasteurianum* was accompanied by sharp decreases in the activities of nitrogenase and glutamate synthase, while glutamine synthetase activity remained

† Present address: Instituto de Investigaciones Pesqueras, Paseo Nacional, s/n, Barcelona-3, Spain.

Fig. 1. Change in the activities of nitrogenase, determined *in vivo* (●) and *in vitro* (○), glutamate synthase (□), glutamine synthetase (■), extracellular NH_4^+ (▲) and bacterial density (A_{660} , △) when sporulation was induced by raising the pH from 6.0 to 8.4 (dashed line).

unchanged (Fig. 1). The rapid and complete inactivation of glutamate synthase should result in an almost complete shut-down in the assimilation of N_2 and NH_4^+ , because NH_4^+ assimilation by glutamate dehydrogenase (EC 1.4.1.2) only occurs at a low level in this organism, if at all (Kleiner, 1979). The inactivation of glutamate synthase contrasts with the behaviour in *A. vinelandii*, where inactivation was not observed (Kleiner & Kleinschmidt, 1976). The less complete inactivation of nitrogenase probably accounts for the massive excretion of NH_4^+ , which cannot be assimilated. Generally a good correlation between NH_4^+ excretion and the difference in the extent of inactivation (nitrogenase activity minus glutamate synthase activity) was observed, irrespective of the method used to induce sporulation.

The question remains unresolved as to whether both enzymes are reversibly inactivated or proteolytically degraded. Enhanced proteolytic degradation during sporulation has been found in several micro-organisms (see Doi, 1972; Holzer *et al.*, 1975; Switzer, 1977) and is likely to operate in *C. pasteurianum* too. Inactivation of the nitrogenase in *A. vinelandii* was accompanied by a rise in the oxygen partial pressure in the culture. It was suggested (Kleiner & Kleinschmidt, 1976) that the first step was inactivation of the enzyme by O_2 , which might be followed by degradation by a non-specific proteinase that degrades denatured proteins in general. As *C. pasteurianum* was maintained in strictly anaerobic conditions during

sporulation, inactivation by O₂ could not occur. Two possibilities may thus be considered. (1) During sporulation non-specific proteinases are formed which are able to degrade all proteins. While some of the proteins are resynthesized (e.g. glutamine synthetase), the synthesis of other proteins is completely or partially repressed (e.g. glutamate synthase and nitrogenase). (2) Induction of the formation of specific proteinases is followed by a specific degradation of nitrogenase and glutamate synthase, while their synthesis continues.

Despite the uncertainties about the mechanism of inactivation, the observations with *A. vinelandii* and *C. pasteurianum* demonstrate the existence of a new regulatory process for N₂ fixation: in addition to controls of activity and synthesis (see Mortenson, 1978) there is a controlled inactivation of the nitrogenase.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (K1 298/9).

REFERENCES

- DOI, R. H. (1972). Role of proteases in sporulation. *Current Topics in Cellular Regulation* **6**, 1–20.
- HITCHINS, V. M. & SADOFF, H. L. (1973). Sequential metabolic events during encystment of *Azotobacter vinelandii*. *Journal of Bacteriology* **113**, 1273–1279.
- HOLZER, H., BETZ, H. & EBNER, E. (1975). Intracellular proteinases in microorganisms. *Current Topics in Cellular Regulation* **9**, 103–156.
- KLEINER, D. (1979). Regulation of ammonium uptake and metabolism by nitrogen fixing bacteria. III. *Clostridium pasteurianum*. *Archives of Microbiology* **120**, 263–270.
- KLEINER, D. & KLEINSCHMIDT, J. A. (1976). Selective inactivation of nitrogenase in *Azotobacter vinelandii* batch cultures. *Journal of Bacteriology* **128**, 117–122.
- MORTENSON, L. E. (1978). Regulation of nitrogen fixation. *Current Topics in Cellular Regulation* **13**, 179–232.
- SWITZER, R. L. (1977). The inactivation of microbial enzymes *in vivo*. *Annual Review of Microbiology* **31**, 135–157.