PROTEOLYTIC ACTIVITY ON AN INSOLUBLE PROTEIN PILED UP BY AN ASPOROGENIC MUTANT OF BACILLUS SUBTILIS

Marvin Rogolsky

Department of Microbiology, College of Medicine

University of Utah, Salt Lake City, Utah 84112

Received June 13, 1968

It appears that one or more proteases activated during post-logarithmic growth are required for the sporulation of Bacillus subtilis (Michel, 1967). Strains unable to produce a late protease are unable to sporulate (Schaeffer, 1967; Spizizen, 1965). A protease appears to be activated during stage zero of sporulation (Schaeffer, 1967) and probably plays an important role in triggering off morphogenesis. Certain asporogenic (Sp^-) mutants which form no protease (PR^-) liberate abundant quantities of a semicrystalline protein after autolysis in Spizizen Minimal Medium (SMM) (Spizizen, 1965). Sp^+ cells also release semicrystalline protein when they are forced to lyse just prior to the onset of sporogenesis but not after they are committed to sporulation. The protein is very insoluble below pH 8 and dissolves with great difficulty in alkali. Although the purpose of the protease in spore formation still remains unknown, experiments executed in this study show that the insoluble protein piled up by Sp^- mutants, blocked at early functions, is hydrolyzed by a late protease produced by Sp^+, PR^+ strains.

Two loopfuls of an overnight culture of a 12A Sp^-, PR^-, BH^-

(Spizizen) mutant on Tryptose Blood Agar Base was inoculated into a 2800 ml Fernbach flask containing 300 ml of SMM fortified with 0.02% Bacto Casitone (Difco) and 1×10^{-5} M $MnCl_2$. After 36 hr of shaking at 37C, the culture autolysed and released insoluble strands of protein. The protein was harvested by centrifugation, washed twice with water and treated with 100 γ/ml lysozyme (Sigma, grade 1) for 45 min at 37C in SMM minus glucose. HCl was added to a final concentration of 0.25N and the mixture remained shaking for an additional 15 min. The protein was sedimented again at 6,000 x g, washed twice in water, suspended into SMM minus glucose and the mixture adjusted to a pH of 7 with 0.1 N NaOH. After a final centrifugation, the protein was deposited into 100 ml of a solution of AK Sporulation Agar (BBL) which was autoclaved and distributed among 4 petri dishes.

One half of a semicrystalline protein plate was streaked with a wild type Marburg strain and the other half with a 12A Sp^-, PR^-, BH^- (Spizizen) strain. After 40 hr of incubation at 37C, wild type cells were able to digest the protein precipitate which was prominently indicated by zones of clearing around Sp^+ colonies growing on semicrystalline protein agar. The 12A Sp^-, PR^-, BH^- colonies were unable to produce zones of clearing (Fig. 1). However, when the Sp^-, PR^-, BH^- strain is made either Sp^-, PR^+, BH^-, Sp^-, PR^+, BH^+, or Sp^+, PR^+, BH^+ by transformation with wild type DNA, it then becomes able to degrade the semicrystalline protein. The PR^+ gene is recognized by the ability of the strain possessing it to form zones of clearing on azo-albumin agar.

The results imply that the semicrystalline protein is an intracellular substrate for a postlogarithmic protease and that events leading

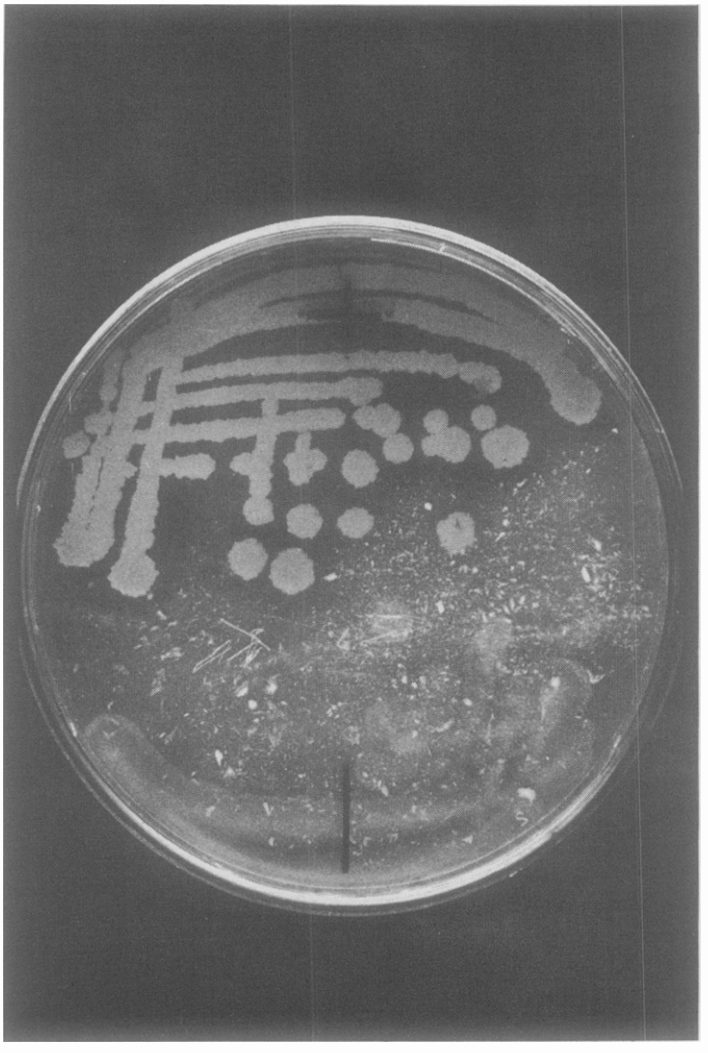

Fig. 1. Colonies of Bacillus subtilis on semicrystalline protein agar. (+) Wild type Marburg colonies surrounded by zones of clearing. (-) Protease defective Sp^- colonies unable to produce zones of clearing.

to sporulation depend upon a rapid turnover of protein which is hydrolyzed by strains with the PR^+ trait. Proteolytic activity on nascent protein probably releases amino acids essential to trigger off many of the mechanisms of sporogenesis. Amino acid concentrations satisfactory for vegetative growth can be insufficient for sporulation of B. subtilis (Jincinska, 1964). Balassa (1964) suggested that amino acids provided by protein degradation regulates the synthesis of spore RNA in B. subtilis. It also appears that sufficient quantities of glutamate are fed into the TCA cycle which is derepressed during sporulation to provide adequate amounts of adenosine triphosphate for morphogenesis (Fortnagel and Freese, 1968). Semicrystalline protein contains more glutamate than any other amino acid (Spizizen, 1965).

ACKNOWLEDGMENT

This work was supported by a grant from the College of Medicine at the University of Utah.

REFERENCES

Balassa, G. Biochem. Biophys. Res. Commun., 15, 236,(1964).

Fortnagel, P. and E. Freese, J. Bacteriol., 95, 1431 (1968).

Jincinska, E., Fol. Microbiol., 9, 72 (1964).

Michel, J., Fol. Microbiol., 12, 297 (1967).

Schaeffer, P., Fol. Microbiol., 12, 291 (1967).

Spizizen, J., Spores III, p. 125 (1965).