Secretion of $\beta$-lactamase by *Escherichia coli* in vivo and in vitro: effect of cerulenin

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The effect of cerulenin on the production of $\beta$-lactamase and other periplasmic proteins was studied in *Escherichia coli* IA199 carrying plasmid pBR322. Cerulenin (10 to 25 $\mu$g/ml) had almost no effect on the growth rate of *E. coli* but it decreased the amount of $\beta$-lactamase and other periplasmic proteins in shock fluid. Higher amounts of the antibiotic (40 to 100 $\mu$g/ml) decreased turbidity and almost completely prevented synthesis of $\beta$-lactamase and other periplasmic proteins. Cerulenin decreased incorporation of L-[^35S]methionine into membranes during growth as well. Spheroplasts secreted $\beta$-lactamase into the external medium, but during a 3-h incubation in the presence of cerulenin (25 $\mu$g/ml) this secretion was prevented by more than 90%. $\beta$-Lactamase was secreted into the isolated membrane vesicles from *E. coli* IA199. However, only 5% of the total amount of pre-$\beta$-lactamase was secreted and processed by the membranes in vitro. Cerulenin did not prevent processing in vitro but the membranes prepared from the cells grown in the presence of cerulenin (25 $\mu$g/ml) did not catalyze processing of pre-$\beta$-lactamase at all. Membrane preparations from *Bacillus subtilis* did not process pre-$\beta$-lactamase either in the absence or in the presence of cerulenin.

**INTRODUCTION**

When the plasmid pBR322 is introduced in *Escherichia coli*, this organism produces a periplasmic $\beta$-lactamase which is a monomeric form of molecular weight 28900 (Ambler and Scott, 1978). The $\beta$-lactamase produced in an in vitro synthesis system in the presence of purified pBR322 plasmid, however, has 23 additional amino acid residues in the amino-terminal end of the protein (Sutcliffe, 1978). This enlarged protein is apparently an unprocessed precursor protein which has not participated in the secretion process, that is a prerequisite
for proteins to become periplasmic. Koshland and Botstein (1980) reported also that β-lactamase is initially synthesized as a complete precursor protein and only after the completion of its synthesis the protein is processed to its final mature length.

The existence of mutants which fail to export periplasmic proteins because of alterations in the amino-terminal signal sequence has been reported (Bassford and Beckwith, 1979; Bedoulle et al., 1980). Furthermore, amber mutations near the carboxyl terminus cause localization of the enzyme in the cytoplasm rather than in the periplasm (Koshland and Botstein, 1980). The signal sequence is thought to be responsible for recognition of the passage site in the membrane and for initiating passage of the polypeptide through the membrane. The signal sequence is enzymatically removed from the polypeptide either before or immediately after translation is completed. Processing of the precursor maltose-binding protein in *E. coli* has been reported to occur post-translationally as well as co-translationally (Josefsson and Randall, 1981). Signal peptidase has been purified 6000-fold from the membranes of *E. coli* (Zwizinski and Wickner, 1980).

Secretion of several bacterial proteins appear to require at least ongoing phospholipid synthesis (Caulfield et al., 1979). Several reports (Fishman et al., 1978; Caulfield et al., 1979; Paton et al., 1980) suggest that inhibition of lipid synthesis by cerulenin inhibits the secretion of extracellular proteins in bacilli. When this manuscript was in preparation Petit-Glatron and Chambert (1981) reported that inhibition of exo-enzyme synthesis by cerulenin in *Bacillus subtilis* is the result of its physicochemical interaction with the membrane rather than interference with intracellular lipid synthesis.

Effects of cerulenin in gram-negative bacteria are very poorly understood. In this paper we present studies of secretion of β-lactamase in *E. coli* IA199 in the presence of cerulenin and production of pre-β-lactamase and its processing *in vitro*.

**MATERIALS AND METHODS**

**Materials**

L-[³⁵S]methionine (1190 Ci/mmol, 44 TBq/mmol) and Na¹²⁵I (carrier free) were from The Radiochemical Centre, Amersham, England. Cerulenin was from Sigma, St. Louis, Mo., U.S.A.

**Bacterial strains and culture conditions**

*Escherichia coli* IA199 carrying the plasmid pBR322 was cultured in ACH-medium containing salts (Vogel and Bonner, 1956), 2 g of glucose, 2 g of Casein amino acids, 1 mg of thiamine hydrochloride and 25 mg of ampicillin per liter. *E. coli* CSH26 and AE1 R⁻ (constitutive for alkaline phosphatase) RNase⁻