SHORT COMMUNICATION

The Characteristics of Extracellular Protein Secretion by
Staphylococcus staphyloyticus

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The differential rate of extracellular protein formation by Staphylococcus staphyloyticus, the lysostaphin-producing organism, was biphasic with a low rate of exoprotein secretion during exponential growth and an increased rate during the post-exponential phase of growth. After 20 h, when no further exoprotein was secreted, exoprotein accounted for 5% of the total protein in the culture. The secretion of three extracellular enzymes was monitored and found to represent a constant proportion of total exoprotein at exoprotein concentrations greater than 0.1 mg ml⁻¹.

INTRODUCTION

The patterns of extracellular protein secretion in several strains of Staphylococcus aureus have recently been studied (Abbas-Ali & Coleman, 1977; Coleman & Abbas-Ali, 1977; Coleman et al., 1978). These investigations have shown a low rate of exoprotein secretion during the exponential growth phase and an increase in secretion as the cultures enter the post-exponential phase of growth. The biphasic nature of exoprotein secretion in these organisms appears to be consistent with the ‘competition’ model for the regulation of exoprotein formation (Coleman et al., 1975) which was originally developed to describe exoprotein secretion by Bacillus amyloliquefaciens.

We report here our studies on the characteristics of extracellular protein secretion by Staphylococcus staphyloyticus, the lysostaphin-producing organism. This organism secretes a staphyloytic glycylglycine endopeptidase, an endo-β-N-acetylglucosaminidase (hexosaminidase), and an acetylmuramic acid-L-alanine amidase, each of which has a site of action on bacterial wall peptidoglycan (Browder et al., 1965; Iversen & Grov, 1973; Wadström & Vesterberg, 1971). We have previously reported that the organism also produces an extracellular sulphhydril protease (Oliver et al., 1976). We have followed the secretion of three of these enzymes (endopeptidase, hexosaminidase and protease) in relation to the growth of the organism and the production of intracellular and extracellular protein in order to determine if this organism also has a pattern of extracellular protein secretion consistent with the ‘competition’ model.

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METHODS

*Staphylococcus staphylolepticus* (NRRL B-2628) was grown in a modification of the lysostaphin production medium described by Zygmunt & Browder (1968). The medium contained (per litre): 60 g enzymic digest of casein (N-Z Amine E, Humko Sheffield Chemical), 5 g soy peptone (Phytone, BBL), 5 g NaCl, 2.5 g K₂HPO₄ and 10 g glycerol. Twenty-nine 250 ml Erlenmeyer flasks each containing 50 ml of lysostaphin production medium were inoculated with a 1% (v/v) inoculum from an 18 h culture of *S. staphylolepticus* in the same medium and incubated at 37 °C with shaking at 250 rev. min⁻¹. At 0 time and every subsequent hour for 28 h a flask was removed, and a 5 ml sample was harvested by centrifuging (12000 g, 10 min) and the supernatant was assayed for endopeptidase, hexosaminidase and protease activities. The dry weight of the bacteria was determined by the method of Stormonth & Coleman (1972). A 20 ml sample from each flask was centrifuged and 10 ml of the supernatant was frozen for later determination of the extracellular protein concentration by the method described by Abbas-Ali & Coleman (1977). The pellet was prepared according to the method of Abbas-Ali & Coleman (1977) for intracellular protein content determinations. All protein determinations were performed using the micro-biuret method according to the procedure of Itzhaki & Gill (1964).

Endopeptidase was assayed as previously described (Robinson *et al.*, 1979). Hexosaminidase was assayed using *Micrococcus lysodeikticus* as the assay organism (Iversen & Grov, 1973; Wadström & Vesterberg, 1971). *Micrococcus lysodeikticus* was grown in Brain Heart Infusion broth (BBL) for 24 h at 30 °C with shaking at 250 rev. min⁻¹. The harvested cells were washed twice with 0.05 M-Tris/HCl buffer (pH 7.2 at 25 °C) and resuspended in the same buffer to an absorbance at 620 nm (A₆₂₀) of 0.250. For the assay, 0.1 ml of an appropriate dilution of culture supernatant from *S. staphylolepticus* was added to 2.9 ml of the *M. lysodeikticus* suspension in 10 × 88 mm cuvettes. After incubation for 10 min in a 37 °C water bath the decrease in absorbance of the reaction mixture was recorded. One unit of hexosaminidase activity was defined as a 50% reduction in turbidity of the standard cell suspension in 10 min. All results were corrected for a cell control to which enzyme was not added.

Protease was assayed using the insoluble proteolotic substrate Azocoll (Calbiochem). The substrate (10 mg) was suspended in 2.5 ml 0.1 m-phosphate buffer (pH 7.1) which contained 1 mM-EDTA, 1 mM-dithiothreitol and 0.5 ml of an appropriate dilution of culture supernatant, and the mixture was allowed to react at 37 °C for 20 min in a shaking water bath at 225 rev. min⁻¹. The reaction was stopped by filtering the mixture through a medium sintered glass filter and the A₅₄₆ of the filtrate in 10 × 88 mm cuvettes was recorded. One unit of protease activity was defined as a change in absorbance of 0.01 units in 20 min. All results were corrected for a substrate control treated in an identical manner except for the addition of enzyme.

RESULTS AND DISCUSSION

The relationship between extracellular protein secretion and growth of *S. staphylolepticus* and the distribution of protein between cellular and extracellular phases are shown in Fig. 1 (a, b). At maximum exoprotein secretion (20 h), exoprotein production in *S. staphylolepticus* accounted for only 5% of the total protein formed, in contrast to *S. aureus* (Wood 46) which has been reported to secrete 33% of its total protein into the extracellular environment (Abbas-Ali & Coleman, 1977).

The patterns of production of the three extracellular enzymes in relation to the growth of *S. staphylolepticus* are shown in Fig. 1 (c). The endopeptidase and protease were detected in the culture medium 1 h before hexosaminidase, presumably because of the more sensitive nature of the respective assays. The concentration of the three enzymes appeared to peak at approximately the same time and then gradually decline. This decline appears to be due to surface denaturation of the enzymes since exoprotein concentration remains constant after 18 to 20 h.

As in other staphylococci studied to date (Abbas-Ali & Coleman, 1977; Coleman & Abbas-Ali, 1977; Coleman *et al.*, 1978), extracellular protein secretion in *S. staphylolepticus* was biphasic, with increased exoprotein formation in the post-exponential phase of growth (Fig. 2a). *Staphylococcus staphylolepticus* showed a 3-1-fold increase in the differential rate of exoprotein secretion at the end of the exponential phase of growth compared with a 4-fold increase in *S. aureus* (Wood 46) (Abbas-Ali & Coleman, 1977). This increase in exoprotein
Fig. 1. (a) Relationship between extracellular protein secretion and the growth of *S. staphylo-lyticus*; ●, bacterial dry weight; ○, extracellular protein. (b) Distribution of protein between cellular and extracellular phases during growth: ●, cellular protein; ○, extracellular protein (the results are expressed as percentages of the total protein produced by the culture after 28 h growth). (c) Production of hexosaminidase, endopeptidase and protease in relation to bacterial dry weight: ●, bacterial dry weight; ○, protease activity; □, endopeptidase activity; ■, hexosaminidase activity.

Fig. 2. (a) Relationship between extracellular protein formation and growth of *S. staphylo-lyticus*; the arrow indicates the bacterial dry weight at 10 h, the end of the exponential phase of growth. (b) Enzyme activity and total extracellular protein secreted during growth: ○, hexosaminidase activity; ●, endopeptidase activity; □, protease activity.
formation has been explained by the ‘competition’ model which suggests that there is competition between intracellular protein and exoprotein production at the level of transcription in rapidly growing cells and that such a competition restricts exoprotein formation almost exclusively to the post-exponential growth phase (Coleman et al., 1975). Extracellular protein secretion in S. staphylolyticus also appears to be consistent with such a model.

The studies on the Wood 46 strain of S. aureus have shown that a graph of α-toxin concentration versus extracellular protein concentration is linear (Abbas-Ali & Coleman, 1977). Since this line passes through the origin the authors suggested a common control mechanism exists for the regulation of exoprotein formation in this organism. For S. staphylolyticus the amounts of endopeptidase, hexosaminidase and protease in the culture supernatant during the period of active extracellular protein secretion (before 16 h, when denaturation of the enzymes became appreciable), as compared to the total extracellular protein concentration in the medium, are shown in Fig. 2(b). The three enzymes each represented a constant proportion of the total extracellular protein at concentrations greater than 0.1 mg ml⁻¹. The linear relationships suggest that a common control mechanism governs the formation of these three enzymes. As further evidence for such a common control mechanism we have found that the production of the three enzymes is not induced when the organism is grown in an amino acid medium, but that all three require the presence of a tryptic digest of casein for their production (Robinson et al., 1979). In addition, mutants of S. staphylolyticus which have lost the ability to produce any one of the three enzymes have invariably lost all three (G. L. Sloan, unpublished results). Such pleiotropic modifications of extracellular enzyme production in bacteria have been reported by several investigators (Forsgren et al., 1971; Omenn & Friedman, 1970; Wretlind et al., 1977; Yoshikawa et al., 1974).

The fact that the lines relating production of the endopeptidase, hexosaminidase and protease to exoprotein concentration do not pass through the origin may indicate that other exoproteins do not share the same control mechanism and that these proteins are secreted prior to the three enzymes investigated. Another possible explanation for these results may be that the ‘extracellular protein’ we detected in the early stages of the growth cycle may actually have been intracellular debris from lysed cells. We have recently shown that there are cells in the population of S. staphylolyticus cultures which are sensitive to the endopeptidase prior to the appearance of the enzyme in the medium (Robinson et al., 1979). As the endopeptidase began to appear in the culture medium these susceptible cells may have been lysed, releasing their intracellular protein. This released intracellular protein would appear to be true exoprotein and would have given artificially high exoprotein concentrations at the onset of exoprotein secretion in the culture.

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REFERENCES


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