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Different responses of *Pseudomonas saccharophila* to induction of α-amylase by starch and by maltose

In previous reports[1,2] we have shown that the extracellular α-amylase (α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1) of *Pseudomonas saccharophila* can be induced in the presence of either starch or maltose. In both cases, the kinetics of appearance of the enzyme in resting cellular suspensions was found to be similar during the first 2–3 h of induction. However, we have observed that when cells are grown overnight on maltose, the final yield of α-amylase is approx 3–5 times greater than that obtained with starch as the carbon source (*cf.* also ref. 3). The present study indicates that the kinetics of enzyme appearance during growth on maltose are quite different from the kinetics obtained with starch.

Cells of *P. saccharophila* were grown in a minimal salts medium containing 0.2% substrate. Further details concerning methods for growing, harvesting, and assaying for α-amylase, reducing sugars, proteins, and starch, have been previously described[1,2,4,5].

When cells were grown with maltose and starch, respectively, results illustrated by Fig. 1 were obtained. In both cases synthesis of total cellular protein was negligible

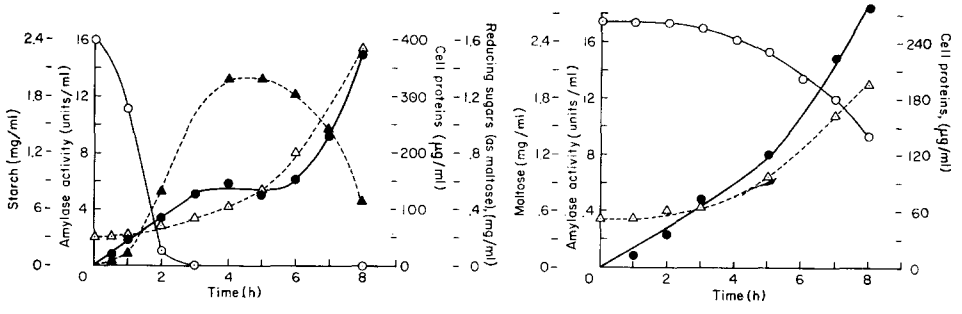

Fig. 1. Kinetics of appearance of α-amylase in starch and maltose cultures. Sucrose-grown cells of *P. saccharophila* were used as the inoculum in both cases. At left, culture growing on starch; at right, on maltose. Open circles, residual substrate; closed circles, amylase activity of supernatants; open triangles, cellular proteins; closed triangles, reducing sugars (assayed as maltose) formed from starch.

during the first 3 h, while amylase appeared in the supernatants at an essentially linear rate during this period (suggesting a preferential synthesis of this enzyme[6]). With maltose as the substrate, enzyme formation then closely paralleled the synthesis of total proteins. By contrast, the kinetics of enzyme appearance with starch-grown cells was discontinuous. After an initial linear rate, there was an interval —a "plateau" phase— during which time, no increase in enzyme was detected in the supernatant fluid. This plateau period generally lasted from 2 to 4 h, after which enzyme appearance in the supernatant was resumed at a logarithmic rate.

That both kinds of cells form the same enzyme is indicated by the fact that the same purification procedure[5] can be used to obtain α-amylase in good yields starting with supernatants from either starch-grown or maltose-grown cells. Furthermore, these enzymes are immunologically indistinguishable in quantitative complement

fixation assays[7]. Using the latter technique, it was also found that the α-amylases formed by starch-grown cells before and after the plateau period are identical.

In order to ascertain the basis for the apparent cessation of enzyme synthesis by starch-grown cells during the plateau period, cells were separated from the supernatant fluid at various times before and during the plateau period. The resultant supernatant fluids were all fully competent to induce fresh cells, and such cultures displayed the same kinetics of enzyme appearance as freshly inoculated starch cultures. It can therefore be concluded that the cessation of amylase appearance in young, starch-grown cultures is not caused by depletion of available inducer, or by the accumulation of an inhibitor in the medium. Nor does enzyme appearance stop simply upon the disappearance of polymeric starch from the medium, for if fresh starch is added at various times before the onset of the plateau period, such cultures exhibit the plateau at about the same time as control cultures. These results thus suggest that the plateau period is the result of some defect in the cells themselves.

In contrast to fresh, uninduced cells, starch-grown cells taken in the plateau period appear to respond only to maltose as an inducer. If starch is added to such cultures after the onset of the plateau period, there is no resumption of enzyme appearance. (Occasionally, a small amount of additional amylase is found upon the addition of starch, but this may represent the release of bound enzyme from the cells.) The addition of maltose, however, immediately results in an increased rate of enzyme appearance (Fig. 2).

The results described above could be explained on the basis of some interference with the secretion of α-amylase in a starch-grown culture as it enters the plateau

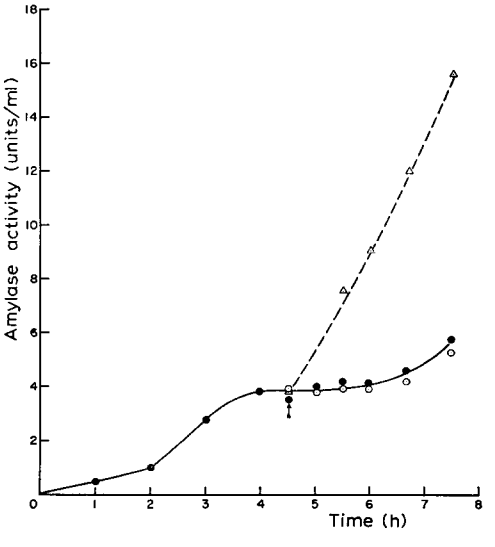

Fig. 2. Effect of added maltose or starch on the plateau period of starch-grown cells. Sucrose-grown cells were inoculated into 0.2% starch plus mineral medium. After the onset of the plateau period (at time indicated by arrow), the culture was divided into three portions; to one, 0.2% (final concentration) starch was added (open circles); to another, 0.2% (final concentration) maltose was added (open triangles); to the third, no further additions were made (closed circles). All three resultant cultures were shaken at 30° and assayed for α-amylase in their supernatant fluids at the times shown.

period. However, a series of experiments, in which cells were sonically disrupted in the growth medium at various times, and the enzymic activity of the resultant suspensions compared with that of similarly sonicated cell-free supernatant fluids, did not support this hypothesis. These experiments showed that there is no increase in the total amylase activity of the culture during the plateau period, although there was usually somewhat more enzymic activity in the whole sonicated culture than in the sonicated supernatant alone. In short, the kinetics of appearance of enzyme in the whole disrupted culture was the same as that of the supernatant fluid alone.

The data given above indicate that cells of *P. saccharophila* grown in maltose behave differently from those grown in the presence of starch. After an initial period of α-amylase formation, starch-grown cells apparently can no longer respond to the presence of oligosaccharides derived from starch, or to starch itself, while retaining their capacity to be induced by maltose. The data seem most readily explained on the basis that the sites of induction (or synthesis) of α-amylase are different for starch and maltose, and that these sites in the case of starch, are lost or destroyed during the initial phase of α-amylase formation. It is possible, for example, that α-amylase is synthesized at or near the cell surface when starch is the inducer, but in the interior of the cell when maltose is used—and that the ribosomes at the cellular surface are more labile than those in the interior. That ribosomes can exhibit different synthetic competencies is suggested by the work of NOVELLI *et al.*[8]. Alternatively, the different effects of starch and of maltose might be attributable to the existence of two separate permeases either of which could allow induction to proceed: one, relatively stable, but specific for maltose, and the other, of broader specificity for higher saccharides and relatively unstable. Separate permeases for the utilization of thio-methyl galactoside and β-methyl galactoside are suggested by the work of ROTMAN in *Escherichia coli*[9]. The authors express their gratitude to the National Science Foundation for partial support (G-6442) of this work, and to Dr. L. LEVINE, Graduate Department of Biochemistry, Brandeis University, for the quantitative complement fixation assays.

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